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A HOLISTIC APPROACH FOR THE FULL VALORIZATION OF FISH WASTE WITHIN CIRCULAR ECONOMY

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Abstract

The linear "production-consumption-disposal" model has resulted in substantial waste generation, causing environmental degradation and resource depletion. In contrast, the circular economy aims to convert waste into valuable resources, minimizing carbon emissions and promoting sustainability. This doctoral thesis aligns with the United Nations' Agenda 2030 for sustainable development, focusing on the valorization of fish processing waste to recover a natural oil rich in omega-3 fatty acids from anchovy fillet leftovers. The study introduces an innovative and sustainable process using *d*-limonene, an edible solvent derived from citrus fruits, in a closed-loop system with full solvent recovery after extraction. Moreover, the solid residue, Anchovy sludge, is further utilized for energy production through anaerobic digestion or as natural fertilizers. Environmental impacts of the entire process were assessed using life cycle assessment models at laboratory and industrial scales. This research not only addresses fish waste valorization but also advocates circular economy practices, reducing the ecological footprint of the fishing industry for a sustainable future.

Chapter 1

Introduction

1.1 Background of the research

The growing awareness of environmental degradation and the depletion of natural resources has led to an increasing focus on sustainable practices in various industries. Among them, the fishing industry is grappling with the challenges of waste generation and its impact on marine ecosystems. In response to these issues, the concepts of the circular economy and blue economy have emerged as promising approaches to foster sustainable development while safeguarding marine biodiversity. This doctoral thesis delves into the nexus between circular economy principles, blue economy strategies, and the valorization of fish waste, specifically exploring the production of fish oil from anchovy fillet leftovers.

The circular economy is a novel paradigm that emphasizes the elimination of waste through the continual circulation of resources in the production and consumption processes. Unlike the traditional linear economy, which follows a "take-make-dispose" model, the circular economy encourages resource regeneration, reuse, and recycling to minimize environmental impact. Within the context of the fishing industry, the circular economy approach advocates for optimizing the entire fish value chain and converting unavoidable waste streams into valuable resources.

In parallel with the concept of the circular economy, the blue economy paradigm encompasses sustainable development strategies centered around marine and coastal resources. It seeks to strike a harmonious balance between economic activities, social inclusion, and environmental protection within ocean-related industries. Embracing a holistic and systemic approach, the blue economy promotes the sustainable management of marine resources, fostering economic growth while safeguarding the oceans for future generations.

The fishing industry is a vital sector that supplies a significant portion of the world's protein intake. However, it also generates substantial amounts of fish waste, including offal, trimmings, and bycatch. Improper disposal of these waste streams can lead to environmental degradation, as decomposition processes consume oxygen and release harmful substances, negatively affecting marine ecosystems and local biodiversity. Additionally, fish waste contributes to greenhouse gas emissions, exacerbating climate change and global warming.

To mitigate the detrimental impacts of fish waste and adhere to circular economy principles, the valorization of fish waste has emerged as an innovative and sustainable approach. Valorization involves transforming waste into valuable products or resources, thereby enhancing the overall efficiency and sustainability of the fishing industry.

One such area of focus is the production of fish oil from anchovy fillet leftovers. Anchovy, a small, oily fish, is abundant in many regions and commonly processed for human consumption.

However, the filleting process generates substantial waste, including heads, tails, and viscera. Instead of discarding these parts, they can be utilized for fish oil extraction.

Fish oil is rich in omega-3 fatty acids, which are essential for human health and have numerous beneficial effects, including cardiovascular protection and cognitive enhancement. The valorization of anchovy fillet leftovers into fish oil contributes to the sustainability of the fishing industry, reduces waste generation, and provides a valuable product for human use.

The primary objective of this thesis is to valorize fish waste through a circular economy approach, particularly focusing on the recovery of fish oil from anchovy fillet leftovers. Fish oil, rich in omega-3 fatty acids, has garnered considerable attention for its health benefits and versatile applications, including pharmaceuticals, nutraceuticals, and aquaculture feeds. By extracting this valuable resource from fish waste, this research not only reduces waste but also contributes to various sectors, promoting a more sustainable blue economy.

The innovative and sustainable process employed in this study centers around the use of *d*-limonene, an edible solvent derived from citrus fruits. This solvent is utilized in a closed-loop system, ensuring complete recovery and recycling after the fish oil extraction process. The closed-loop system is a key aspect of circular economy principles, as it minimizes waste generation and fosters resource efficiency.

In addition to fish oil extraction, this research recognizes the value of the solid residue, known as Anchovy sludge, which results from the process. The study explores two significant valorization pathways for Anchovy sludge. Firstly, through anaerobic digestion, the sludge can be converted into biogas, a renewable energy source that can substitute conventional fossil fuels, thereby contributing to a reduction in greenhouse gas emissions. Secondly, the by-products of the extraction process can be transformed into natural fertilizers, closing nutrient loops and promoting sustainable agriculture.

To comprehensively assess the environmental impacts of the entire fish waste valorization process, life cycle assessment (LCA) models were applied at both laboratory and industrial scales. LCA provides a holistic analysis of the process's environmental footprint, considering inputs, outputs, and potential impacts at each stage. Through LCA, this research ensures that the proposed circular approach is environmentally viable and aligned with the objectives of sustainable development.

The dissertation is divided into six main chapters.

The first chapter of this thesis introduces the concepts of the circular economy and the blue economy, highlighting their importance in fostering sustainable practices within the fishing industry. Emphasis is placed on the valorization of fish waste, with a particular focus on anchovy fillet leftovers. This chapter includes a comprehensive description of the global anchovy market, examining its significance in Europe and specifically in Italy, which is among the prominent producers and consumers of anchovy products.

Furthermore, the chapter explores the prospect of sustainable and zero-impact fish oil production from the discarded anchovy fillet leftovers. Rich in omega-3 fatty acids, fish oil possesses numerous health benefits, making it a valuable resource for pharmaceuticals, nutraceuticals, and aquaculture feeds. The importance of omega-3 consumption in human diets is also emphasized, along with an overview of the omega-3 market. Additionally, the chapter delves into the production of biofertilizers derived from fish waste, highlighting the potential for generating value from this previously overlooked resource.

The second chapter elaborates on the development of an innovative and sustainable process for valorizing anchovy waste and obtaining fish oil rich in omega-3 through simple extraction with *d*-limonene. Detailed analyses and comparisons are conducted with other oils extracted using organic solvents and conventional methods, demonstrating the comparable quality of the oil extracted using *d*-limonene. This chapter underscores the efficacy of the proposed process in aligning with circular economy principles and advancing sustainable fish waste valorization.

In the third chapter, attention is directed towards one of the valorization routes for the solid residue generated during fish oil extraction, known as Anchovy sludge. This residue is utilized as a substrate for biogas production through anaerobic digestion. The chapter presents experimental analyses, including batch and semi-continuous tests, highlighting the validity of Anchovy sludge as a co-digestion substrate. The conversion of Anchovy sludge into biogas represents a crucial step towards renewable energy generation and waste reduction.

The fourth chapter explores the second valorization route for Anchovy sludge, its utilization as organic fertilizer. The chapter includes experimental tests evaluating its fertilizing activity in the cultivation of red Tropea onions, comparing its performance with commercial NPK fertilizer and organic manure. This chapter underscores the potential of Anchovy sludge as an environmentally friendly alternative for enhancing agricultural productivity.

Finally, in the fifth chapter, the thesis employs the life cycle assessment (LCA) methodology to comprehensively evaluate the environmental impacts of the entire process, both at the laboratory and scale-up levels. The LCA analysis provides critical insights into the overall sustainability of the proposed fish waste valorization approach. The thesis concludes with final reflections and conclusions, summarizing the findings and their implications for advancing circular economy practices and reducing the ecological footprint of the fishing industry.

1.2 Blue economy

The concept of the blue economy, also known as the ocean or maritime economy, revolves around the responsible and sustainable utilization of ocean resources to foster economic growth, improve livelihoods, and safeguard ocean health. The blue economy encompasses a diverse array of sectors, including fisheries, aquaculture, shipping, energy, tourism, and marine biotechnology. Policymakers, scholars, and stakeholders have increasingly recognized its potential to drive sustainable development and alleviate poverty [1].

Despite the promising prospects, the blue economy encounters various contemporary challenges that pose threats to its sustainability and potential benefits. Climate change, overfishing, pollution, and habitat destruction emerge as significant menaces, directly impacting the health of our oceans and their resources. These challenges not only harm the environment but also bear economic and social repercussions, such as the loss of biodiversity, livelihoods, and cultural heritage [2].

From an academic perspective, the blue economy necessitates a multidisciplinary approach that integrates natural, social, and economic sciences to comprehend the intricate interplay between human activities and ocean ecosystems. Scholars have underscored the significance of policy coherence, effective governance frameworks, and active involvement of stakeholders to ensure the sustainable utilization of ocean resources. Recent literature has also accentuated the role of innovation, technology, and financial support in facilitating the transition towards a blue economy [3].

In conclusion, the blue economy presents both opportunities and challenges that call for a comprehensive and interconnected approach to strike a balance between economic growth, environmental sustainability, and social equity.

1.2.1 Blue economy origins

The concept of the blue economy traces its roots back to the early 2000s, a time when global interest in sustainable development was gaining momentum. Gunter Pauli, a Belgian entrepreneur and advocate for sustainability, is credited with coining the term "blue economy." He introduced this idea in his book titled "The Blue Economy: 10 Years, 100 Innovations, 100 Million Jobs," which was published in 2011 [1]. Pauli's book put forth the concept of a novel economic model that revolves around the efficient and sustainable utilization of ocean resources. He argued that the oceans hold significant untapped wealth and have the potential to address numerous environmental and economic challenges faced by the world, including issues like climate change, energy scarcity, and poverty [4].

Pauli's vision introduced a groundbreaking business model that emulates natural systems and derives value from waste and by-products. His emphasis on sectors like aquaculture, renewable energy, and biotechnology underscored their potential to foster economic growth while concurrently promoting environmental sustainability and social inclusivity [4].

Since its inception, the concept of the blue economy has garnered widespread recognition from influential international organizations like the United Nations and the World Bank. Additionally, governments, academia, and the private sector have embraced this idea as a pivotal driver of sustainable development, offering a pathway to achieving the United Nations' Sustainable Development Goals (SDGs). Notably, SDG 14, which centers on the conservation and sustainable use of oceans, seas, and marine resources, aligns closely with the principles of the blue economy [5].

Indeed, Gunter Pauli's contribution to the blue economy concept is significant, but it is important to recognize that other influential thinkers and initiatives have also played a crucial role in shaping its evolution.

One such milestone was the 1992 United Nations Conference on Environment and Development (UNCED) held in Rio de Janeiro, Brazil. During this conference, the adoption of Agenda 21 took place, which was a comprehensive plan of action for promoting sustainable development. Agenda 21 recognized the vital role of oceans, seas, and coasts in the pursuit of sustainable development. It emphasized the need for integrated management and sustainable use of ocean resources and ecosystems [6].

Additionally, the 2002 World Summit on Sustainable Development (WSSD) held in Johannesburg, South Africa, further emphasized the significance of the ocean economy as a catalyst for economic growth and poverty reduction. As a result of the WSSD, the Global Programme of Action for the Protection of the Marine Environment from Land-based Activities was established. This program aimed to mitigate the impacts of human activities on the marine environment, highlighting the importance of safeguarding marine ecosystems [7].

Together, these conferences and initiatives have contributed to the development and recognition of the blue economy concept as a vital framework for achieving sustainable development goals while ensuring the responsible use of ocean resources.

Indeed, the blue economy has garnered significant momentum as a pivotal strategy for achieving sustainable development. Various regions and international bodies have embraced this concept, recognizing its potential to promote environmental sustainability, economic growth, and social development.

For instance, the European Union has developed a blue growth strategy that centers on the sustainable utilization of marine resources. This strategy aims to balance economic development with responsible resource management to ensure long-term benefits [8].

Similarly, the African Union has launched its Blue Economy Strategy, with a focus on fostering sustainable economic growth, ensuring food security, and creating job opportunities through the development of marine resources [9].

Overall, the blue economy concept has emerged from and is deeply rooted in the broader sustainable development agenda. It has evolved into a critical pathway that aligns economic activities with environmental conservation, leading to a more sustainable and equitable future. As governments and international bodies continue to endorse and implement blue economy strategies, there is hope for achieving a harmonious balance between economic progress and the protection of our oceans and marine ecosystems.

1.2.2 Blue economy dimensions

In contemporary research, the blue economy is commonly understood to encompass six dimensions, each characterized by principles and practices aimed at ensuring the sustainable utilization of ocean resources for the benefit of present and future generations [10][11].

1. Economic Dimension

The economic dimension of the blue economy revolves around fostering economic growth and development while prioritizing sustainability. It encompasses diverse activities such as fishing, aquaculture, marine biotechnology, tourism, shipping, and renewable energy. The principles of this dimension emphasize creating value from ocean resources, driving innovation, and establishing sustainable business models that support local communities.

2. Social Dimension

The social dimension of the blue economy focuses on equitable distribution of benefits from ocean resources among all stakeholders. It addresses various social concerns, including human rights, labor standards, gender equality, and community development. The principles of this dimension center on building partnerships, engaging with local communities, and considering the social impacts of economic activities.

3. Environmental Dimension

The environmental dimension of the blue economy aims to safeguard the health and integrity of ocean ecosystems. It addresses matters such as biodiversity conservation, climate change mitigation and adaptation, pollution prevention, and ecosystem-based management. The principles of this dimension stress the importance of ensuring economic activities do not compromise the ecological sustainability of ocean resources.

4. Technological Dimension

The technological dimension of the blue economy is focused on developing and applying new technologies to support sustainable economic activities in the ocean. This encompasses areas like marine robotics, sensors, artificial intelligence, renewable energy technologies, and biotechnology. The principles of this dimension encourage innovation, responsible technology transfer, and sustainable use of emerging technologies.

5. Cultural Dimension

The cultural dimension of the blue economy involves recognizing and preserving the cultural heritage and traditional knowledge related to ocean resources. It includes practices such as fishing, seafaring, storytelling, and cultural tourism. The principles of this dimension emphasize respecting and valuing cultural diversity, recognizing the cultural dimensions of ocean resources, and promoting cultural exchange and cooperation.

6. Governance Dimension

The governance dimension of the blue economy deals with establishing effective governance mechanisms that support the sustainable management of ocean resources. It addresses issues like institutional frameworks, policy coordination, and stakeholder engagement. The principles of this dimension focus on creating transparent, participatory, and accountable governance mechanisms that promote the sustainable use of ocean resources.

Research on the blue economy aims to explore the interconnections between these dimensions and identify pathways for promoting sustainable development that strikes a balance between economic growth, environmental protection, and social well-being. Key research areas include ocean governance, marine policy, ecosystem services, climate change adaptation, and sustainable business models. Modern blue economy research aims to create a comprehensive framework that supports the sustainable use of ocean resources for the benefit of both present and future generations.

1.2.3 The effect of the blue economy on the world economy

The blue economy's potential impact on the global economy has garnered significant attention and several estimates and projections have been made to highlight its significance [12].

According to a report by the Organisation for Economic Co-operation and Development (OECD) in 2016, the ocean economy already contributed approximately USD 1.5 trillion to the global economy, equivalent to around 2.5% of the worldwide GDP. The report also projected that the ocean economy has the potential to double its contribution by 2030 if appropriate policies and investments are implemented. Emerging sectors such as offshore wind energy, aquaculture, and marine biotechnology were identified as key drivers of economic growth and job creation [13].

In a more recent report by the World Wildlife Fund (WWF) in 2019, it was estimated that the blue economy could generate up to USD 3 trillion in value and create as many as 40 million jobs by 2030. This report emphasized the importance of pursuing a sustainable blue economy that balances economic growth with environmental and social sustainability. Sustainable fishing, coastal tourism, and marine renewable energy were highlighted as potential areas that could create significant economic opportunities [14].

These reports underscore the substantial economic potential of the blue economy and the importance of adopting sustainable practices to maximize its benefits while ensuring the preservation of marine ecosystems and the well-being of communities reliant on ocean resources. With the right policies and investments, the blue economy can be a significant driver of economic growth and job creation while contributing to the broader goals of environmental protection and social development.

Indeed, the blue economy's potential contributions to the global economy are substantial, and various studies have provided valuable insights into its economic significance.

According to the European Commission's study in 2019, the blue economy had already generated approximately 5.4 million jobs and contributed around EUR 750 billion to the EU economy in 2018. The study further projected that with the implementation of appropriate policies and investments, the blue economy's contribution to the EU economy could reach up to EUR 1.3 trillion by 2030 [15].

These estimations and projections highlight the economic benefits that the blue economy can bring if it is developed in a sustainable and responsible manner. However, it is essential to recognize that the impact of the blue economy goes beyond mere economic output. Its significance is also measured in terms of the social and environmental advantages it can offer, such as enhancing food security, reducing poverty, and preserving marine biodiversity.

As the blue economy continues to evolve, it is crucial to strike a careful balance between economic growth and the preservation of the marine environment and the well-being of coastal communities. By adopting sustainable practices, responsible management, and inclusive policies, the blue economy can thrive while ensuring the long-term health and resilience of our oceans and their resources. This approach will maximize its benefits not only for the economy but also for society and the environment as a whole.

The blue economy indeed holds great potential to significantly impact the economies of Middle Eastern countries, given the region's extensive coastline, access to the Red Sea and Arabian Gulf, and rich marine biodiversity. Here are some ways in which the blue economy is expected to affect the Middle East, based on research [16]:

- Fishing and Aquaculture: The Middle East has a longstanding tradition of fishing and seafood consumption. With access to the Red Sea and Arabian Gulf, the region offers diverse fish and seafood resources. The Food and Agriculture Organization (FAO) reports that aquaculture production in the Middle East and North Africa (MENA) region increased from 170,000 tons in 1990 to 1.4 million tons in 2018, indicating significant potential for economic growth in this sector [17].

- Marine Tourism: The Middle East is home to some of the world's renowned and luxurious coastal resorts, attracting millions of tourists annually. The region's rich marine biodiversity and natural beauty create opportunities for recreational activities like snorkeling, diving, and boat tours. The World Travel and Tourism Council (WTTC) reports that travel and tourism contributed USD 196 billion to the MENA region's GDP in 2019, with further growth expected through the development of marine tourism [18].
- Offshore Oil and Gas: The Middle East is a significant producer of oil and gas, and offshore exploration and production play a crucial role in the region's economy. The Persian Gulf region alone accounted for approximately 28% of the world's crude oil production and 20% of its natural gas production in 2020, according to the U.S. Energy Information Administration (EIA). Developing new offshore oil and gas fields and expanding existing areas can further boost the region's economic prospects [19].
- Renewable Energy: The Middle East is well-positioned to harness renewable energy from marine sources like wind, wave, and tidal power. The region's vast coastlines and exposure to strong winds and currents offer substantial potential for renewable energy generation. The International

Renewable Energy Agency (IRENA) estimates that offshore wind energy potential in the region could reach 3.6 GW by 2030, creating jobs and contributing to carbon emission reduction efforts [20].

In summary, the blue economy presents various economic growth and diversification opportunities in the Middle East, particularly in sectors such as fisheries and aquaculture, marine tourism, offshore oil and gas, and renewable energy. By capitalizing on these opportunities in a sustainable and responsible manner, Middle Eastern countries can further enhance their economic prosperity while safeguarding the marine environment and promoting social development.

Absolutely, to fully realize the potential benefits of the Blue Economy, countries in the Middle East and beyond must address various challenges related to overfishing, pollution, and climate change. Adopting policies and strategies that promote sustainable and responsible use of marine resources is crucial for the long-term health and productivity of oceans.

The Blue Economy presents several positive effects on businesses, including:

- Diversification of Economic Activities: Embracing the Blue Economy allows businesses to expand beyond traditional sectors and explore new markets in marine-based renewable energy, biotechnology, and marine tourism. This diversification can lead to new revenue streams and job creation, contributing to economic growth and resilience [21].

- Innovation and Technological Advancement: The Blue Economy encourages innovation and the adoption of new technologies to support sustainable ocean resource use. This fosters the creation of new businesses and products/services that cater to the needs of ocean users, driving economic growth and competitiveness [22].
- Increased Market Access: Engaging in the Blue Economy provides businesses with access to a larger market for ocean-related products and services, both locally and globally. This expanded market access can boost sales, revenue, and overall business growth [23].
- Improved Reputation: Businesses that embrace sustainable practices within the Blue Economy can enhance their reputation and brand image. As consumers increasingly seek to support environmentally responsible companies, adopting sustainable practices can lead to increased customer loyalty and market share [24].
- Collaboration and Partnerships: The Blue Economy encourages partnerships among businesses, governments, and civil society organizations to promote sustainable ocean use. Such collaborations foster knowledge sharing, expertise exchange, and

resource pooling, benefitting businesses while advancing sustainable development [25].

In conclusion, the Blue Economy holds immense potential to positively impact businesses by offering opportunities for diversification, innovation, increased market access, improved reputation, and collaboration. However, it is essential for companies to adopt sustainable practices within the Blue Economy to ensure the long-term health and productivity of ocean resources, creating a win-win situation for both businesses and the environment.

1.2.4 Blue economy challenges

To unlock the vast potential benefits of the Blue Economy, nations in the Middle East and other regions must tackle a range of challenges related to overfishing, pollution, and the impact of climate change on marine ecosystems.

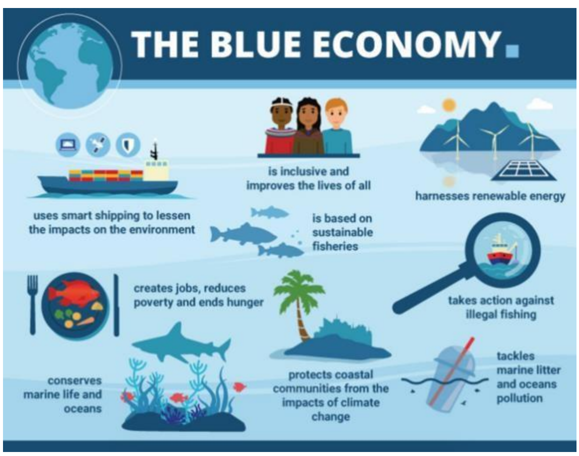

Figure 1.1 Development of the Blue economy (https://escaribbean.com/blue-economy-harnessing-blue-benefits/).

It is imperative to implement policies and strategies that prioritize the sustainable and responsible utilization of marine resources, safeguarding the long-term health and productivity of our oceans.

The Blue Economy (Figure 1.1) offers numerous positive effects on businesses, including:

- Economic Diversification: Embracing the principles of the Blue Economy enables businesses to expand beyond traditional sectors and explore new opportunities in marine-based renewable energy, biotechnology, and marine tourism. This diversification can lead to the generation of fresh revenue streams and job opportunities, fostering economic growth and resilience [21]
- Fostering Innovation and Technological Advancement: The Blue Economy fosters a culture of innovation and the adoption of cutting-edge technologies to support sustainable ocean resource use. This, in turn, spurs the creation of novel businesses, products, and services that cater to the needs of ocean users, driving economic growth and enhancing competitiveness [22].
- Access to Expanded Markets: Engaging in the Blue Economy grants businesses access to a broader market for ocean-related products and services, both at a local

and global scale. This expanded market access can boost sales, revenue, and overall business growth [23].

- Enhanced Reputation: Companies that embrace sustainable practices within the Blue Economy can significantly improve their reputation and brand image. As consumers increasingly seek to support environmentally responsible companies, adopting sustainable practices can lead to increased customer loyalty and market share [24].
- Promoting Collaboration and Partnerships: The Blue Economy encourages collaboration among businesses, governments, and civil society organizations to promote sustainable ocean use. Such partnerships foster knowledge sharing, expertise exchange, and resource pooling, benefiting businesses while advancing sustainable development [25].

In conclusion, the Blue Economy holds immense potential to positively impact businesses by offering opportunities for economic diversification, innovation, increased market access, improved reputation, and collaborative growth. However, it is crucial for companies to embrace sustainable practices within the Blue Economy to ensure the long-term health and productivity of ocean resources, creating a mutually beneficial relationship for both businesses and the environment.

The future of the Blue Economy holds great promise as global interest in sustainable ocean development continues to grow, recognizing the ocean's economic value. Several key trends are shaping the path forward for the Blue Economy:

- Embracing Sustainable Practices:

There is a growing realization that adopting sustainable practices is crucial for the long-term viability of the Blue Economy. Ecosystem-based management and circular economy principles are gaining traction as essential strategies.

- Technological Advancements:

Emerging technologies such as robotics, autonomous systems, and advanced sensors are being harnessed to enable sustainable ocean development. These innovations have the potential to enhance efficiency and productivity while minimizing adverse impacts on the marine environment.

- Ocean-Based Renewable Energy:

The exploration of ocean-based renewable energy sources, such as offshore wind and wave energy, is on the rise. This sector not only offers new employment opportunities but also addresses the escalating demand for clean and renewable energy.

- Circular Economy Practices:

The concept of a circular economy is gaining prominence within the Blue Economy. By maximizing resource efficiency and implementing practices that prioritize product reuse, recycling, and remanufacturing, waste generation can be minimized.

- Sustainable Tourism:

Sustainable tourism is becoming increasingly popular, and there is a heightened interest in developing marine tourism that supports responsible ocean resource utilization.

- Advancements in Blue Finance:

Blue finance, characterized by innovative financial instruments, is attracting growing interest. Impact investing, green bonds, and other sustainable finance models are being utilized to support and finance sustainable ocean development initiatives.

In conclusion, the future of the Blue Economy appears promising, driven by the increasing focus on sustainable practices, technological innovation, ocean-based renewable energy, circular economy principles, sustainable tourism, and blue finance. The development of these sectors presents significant opportunities for collaboration between businesses, governments, and civil society, enabling sustainable development while safeguarding the health of our oceans. By working together, we can ensure a prosperous and sustainable future for the Blue Economy.

1.2.5 Fish waste in the circular bioeconomy era

The substantial growth in global population over the last two decades, coupled with the extensive utilization of finite resources, has had detrimental effects on the environment, prompting the need for sustainable strategies. To secure a better future for generations to come, it is crucial to explore alternative resources that can replace fossil fuels and foster the development of renewable processes grounded in sustainability. A vital aspect of managing resources in an eco-efficient manner involves transitioning from a linear economy to a circular one, as sustainability relies on the circularity of essential materials.

Integral to the concept of the circular economy is the circular bioeconomy, which plays a pivotal role in achieving resource and environmental sustainability. The bioeconomy revolves around the utilization of materials of biological origin and the emulation or adaptation of nature's processes to ensure efficient resource utilization [26]. As defined by the European Commission, the bioeconomy encompasses "the production of renewable biological resources and the conversion of these resources and waste streams into value-added products, including food, feed, bio-based products, and bioenergy" [27].

Thus, by embracing the circular bioeconomy, human reliance on fossil-derived raw materials diminishes, while renewable resources gain support, leading to a minimized environmental impact. Several key strengths define the circular bioeconomy's potential, including increased awareness among individuals and industries, active engagement of stakeholders and policymakers, political backing, sustainable production and consumption practices, resource valorization, and the pursuit of zero waste.

The fundamental role of bio-waste valorization becomes evident in establishing circularity within the bioeconomy. This domain has captured significant attention from the scientific community, with considerable government support, dedicated to recovering resources from biological waste. Notably, efforts are directed towards utilizing unwanted marine resources, like the substantial waste generated from fishing and aquaculture, which shows great promise for yielding high-value products [28][29][30].

Regrettably, each year, a vast amount of biomass is either discarded, often incinerated, resulting in increased energy consumption, financial costs, and environmental impact during their management process [31], or it is utilized for low-value products. Presently, fish waste mainly finds application in the fish meal industry due to its protein content being comparable to fish meat [32][33][34].

Additionally, the nutritional composition of fish waste offers the opportunity to supply essential plant nutrients or enrich compost. Fish waste can be processed to create various fertilizers [35], and currently, commercial fish-based fertilizers find applications in agriculture and horticulture for crops [36]. Moreover, fish waste exhibits a high concentration of biodegradable organics, making it an attractive co-substrate for waste activated sludge during anaerobic co-digestion, thereby enhancing methane production [37,38].

Fish processing is essential for large fish companies to reduce costs related to the transport of inedible fish parts and to enhance product stability and quality. Removing parts like viscera becomes necessary as they may contain bacteria and enzymes, posing risks during fish processing and storage [39]. Preserving the nutritional quality of products remains a significant challenge for the industry. Minimizing the degradation of proteins by enzymes is critical, as excessive hydrolysis can lead to the formation of bitter-tasting peptides [39][40]. Additionally, lipid peroxidation can cause variability in raw materials [40][41], making acceptable levels of lipid and protein hydrolysis dependent on the product and its intended use [42][43].

Hydrolysis, responsible for structural and conformational changes in proteins, can have adverse effects on their physicochemical and functional properties [39]. Therefore, controlling the autolysis and auto-oxidation of these products becomes crucial for their use [44]. This presents a significant challenge for fishing vessels, necessitating advanced equipment and technologies for capture and handling to maintain the quality of the byproducts [45][46].

Furthermore, to ensure food safety and consumer protection, increasingly stringent hygiene measures have been implemented at both the national and international levels. However, a substantial amount of by-catch is rejected each year, comprising low-value species and tons of commercially valuable yet undersized fish. The quantity of fish byproducts is expected to rise in the coming years due to the implementation of the landing obligation, as part of the new reform of the EU Common Fisheries Policy (CFP) [47][48]. The landing obligation requires the mandatory retention of all commercially exploited species within the total allowable catch regulations, including undersized fish that cannot be used for direct human consumption and endangered species. This measure aims to combat the wasteful practice of fish discards, ensuring that precious fish biomass is not needlessly wasted [47][48]. Alarmingly, approximately 25% of all caught fish never reaches the market, with roughly 27 million tons of unwanted fish discarded into the sea each year, much of which does not survive.

The new landing obligation's ultimate objectives align closely with two other EU strategies, namely Blue Growth and the 2020 EU Strategy, both dedicated to promoting sustainable socio-economic and environmental growth in the marine and maritime regions of the EU [49].

Blue Growth is a forward-looking strategy that centers around harnessing the potential of seas and oceans for innovation and economic expansion, providing a pathway for the EU to navigate its current challenges and drive economic prosperity. The EU's blue economy encompasses a wide range of activities related to the sea, generating 5.4 million jobs and contributing a gross added value of nearly €500 billion annually. The Organization for Economic Co-operation and Development (OECD) predicts that numerous ocean-based industries have the potential to outperform the global economy by 2030, both in terms of value added and job creation. In fact, the output of the global ocean economy could more than double by that time [50].

A key component of the Blue Growth strategy focuses on blue biotechnology, which involves the transformation of raw marine materials into high-value products with various biotechnological applications. This approach fosters the development of innovative markets, aligning with the objectives of EU strategies. In this context, valorization strategies for fish discards and byproducts can significantly contribute to economic growth. By finding new uses for fish waste, the burden of the landing obligation can be eased, and the environmental challenges associated with managing vast amounts of waste can be mitigated.

In summary, the Blue Growth strategy recognizes the immense potential of marine resources and aims to leverage them for sustainable economic advancement, with blue biotechnology and the valorization of fish waste playing essential roles in achieving these goals.

1.3 Fish waste valorization: a path towards sustainable fisheries

In the context of sustainable natural resource management, marine-derived bioresources with commercial value have become a focal point. The 2030 agenda for the United Nations' sustainable development goals (SDG Target 12.3) recognizes fisheries as an essential feedstock, not only for food security and nutrition but also for their broader importance.

Fishing, one of the oldest human activities, continues to play a crucial socioeconomic role in numerous countries worldwide. In 2020, the combined efforts of fishing and aquaculture yielded an impressive 178 million tons of aquatic animals, showing a steady compound annual growth rate of 2-3.5%, generating approximately US$ 406 billion in revenue and providing employment to around 58.5 million people in primary production [51]. Notably, Asian countries accounted for about 70% of the total fishing and aquaculture production, followed by American countries with 12%. Among the aquatic production, around 76% comprises various fish species, whether from marine or freshwater sources. The primary fish species produced include carp, with Ctenopharyngodon idellus being a prominent variety, followed by barbers, herring, sardines, and anchovies [51].

Fish consumption has significantly increased in recent years, however, as the demand for fish protein rises, so does the amount of waste generated. It is estimated that meeting the market demand results in the production of up to 20 million tons of waste in the fish industry [52]. Despite efforts to maximize the utilization of edible fish products, waste and by-products still constitute nearly 75% of the fish body weight.

Fish is a valuable and nutritious source of animal protein, contributing to approximately 17% of the world's animal protein intake [53]. The composition of fish varies depending on factors such as age, sex, health, species, and season [54]. As the global population continues to grow, the demand for aquatic food is projected to increase to meet protein requirements [55].

However, despite the increase in fish utilization, the generation of waste remains a significant concern due to its perishable nature and the presence of inedible parts. Fish processing, whether at domestic or industrial scale, results in considerable fish discards. Solid fish waste is a heterogeneous and potentially hazardous byproduct if not properly treated in fish farms and post-harvest systems. This waste includes bones, heads, skin, scales, fins, tails, gut, and viscera in varying proportions (Figure 1.2).

Unfortunately, a considerable amount of this organic-rich waste is dumped in landfills and water bodies, posing environmental threats through land and water contamination. Improper handling and storage practices can rapidly deteriorate fish quality, rendering them unfit for consumption. These issues are often exacerbated by factors such as exposure to high temperatures for extended periods, inadequate infrastructure, and reliance on traditional post-harvest fish processing methods that lead to high levels of wastage [57].

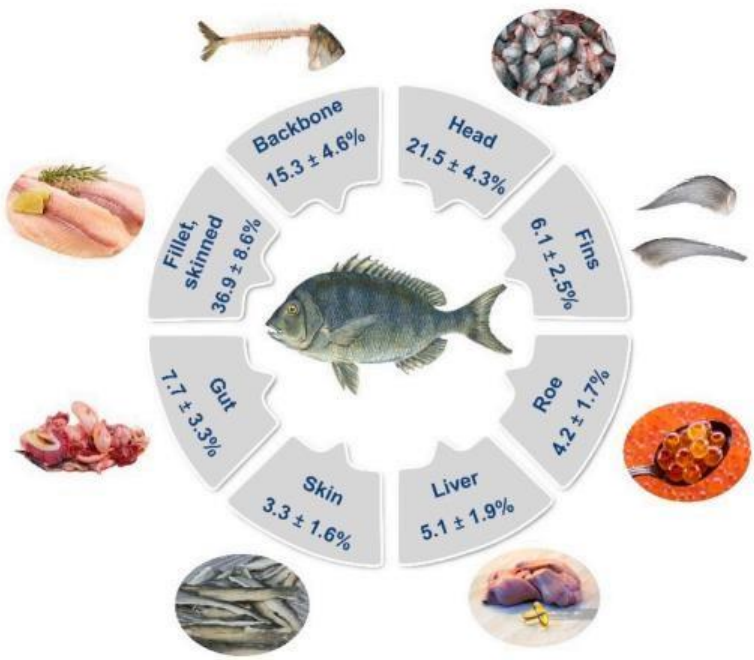

Figure 1.2 Major fish by-products. Values are an average of different fish species (by percentage body weight) and on dry basis, adapted from [56].

Efforts are needed to minimize fish wastage through scientific practices and prioritize the valorization of fish discards in solid waste management. Recovering valuable components from fish waste before disposal has become a crucial objective in sustainable waste management strategies. By addressing these challenges, we can move towards more sustainable and responsible management of marine-derived bioresources and contribute to the achievement of broader sustainable development goals.

On a global scale, the management of solid fish waste presents several challenges, leading to various disposal methods that can potentially impact the environment and human health.

Common practices include incineration, composting, anaerobic digestion, landfilling, sea dumping, and even abandonment. Each of these methods comes with its set of environmental implications.

Addressing the challenge of fish waste and by-products is crucial to ensure sustainability in the fishing industry. Developing innovative methods to utilize these by-products efficiently can lead to significant environmental and economic benefits, promoting a more responsible and resource-efficient approach to fish production and consumption. Indeed, the fish parts that are often discarded as waste, such as the skin and fins, contain a significant amount of collagen, which can be converted into valuable gelatin [58]. Additionally, the heads and viscera of fish are rich in oil, particularly polyunsaturated fatty acids, and are a valuable source of fat-soluble vitamins A and D. This makes them highly promising for oil extraction applications, especially in the food and pharmaceutical industries, as fish can have a fat content of up to 45% of their body composition [59]. Fish oils obtained from these waste parts, which may not be suitable for direct human consumption, can be repurposed for the production of biodiesel [60][61].

Furthermore, fish waste offers an opportunity to extract astaxanthin, a potent antioxidant compound with various health benefits [62[[63][64]. This value-added compound has potential applications in the food and nutraceutical industries.

By harnessing these valuable components from fish waste, we can significantly reduce the environmental impact of discarding these by-products and create new avenues for sustainable resource utilization. The extraction and utilization of gelatin, fish oils, and astaxanthin from fish waste represent promising opportunities for the development of innovative and eco-friendly products, benefiting both the fishing industry and the broader market. The extraction of gelatin from fish waste has been commonly reported in the literature, and traditional methods often involve the use of hazardous chemicals such as HCl and NaOH [65][66][67], as well as solvents like petroleum ether and hexane [68][69[61]. While these methods are efficient in obtaining gelatin, they result in the production of hazardous effluents, posing significant challenges for the ecosystem. Therefore, it is essential to explore more environmentally friendly and sustainable approaches to extract and recover value-added compounds like collagen, gelatin, and oil from solid fish waste before disposing of them. Adopting scientific practices and embracing the principles of the circular economy can expand the possibilities for the recovery of valuable compounds from fish waste in a more eco-friendly manner [70][71][72]. By utilizing less aggressive and more sustainable extraction and purification methods, we can minimize environmental impacts while still deriving valuable products from fish waste.

However, one traditional approach to address this issue is the production of biogas through anaerobic digestion of solid fish waste, often combined with co-substrates such as animal manure [73]. Studies have shown that adding solid fish waste at a 5%

concentration leads to higher biochemical methane production, with a similar trend observed for high-fat fish species like tuna, sardine, and needlefish [74].

Moreover, the anaerobic digestion process yields a commercially viable byproduct: bio-fertilizers derived from the digestate. These bio-fertilizers can serve as valuable agricultural inputs. Another promising application of fish by-products and by-catches is the extraction of fish oil, which generally possesses high hydrogen and low carbon content, making it suitable for biodiesel production [75].

By adopting such innovative and sustainable waste management practices, we can mitigate the adverse environmental impacts associated with solid fish waste disposal while simultaneously creating valuable products and reducing the burden on traditional waste treatment methods. However, further research and implementation efforts are necessary to promote these techniques on a broader scale and contribute to a more environmentally friendly and resource-efficient approach to managing fish waste.

The utilization of fish by-products and discards presents a significant challenge that requires the adoption of appropriate downstream processes targeting specific components. Over the years, various conventional techniques have been employed to extract valuable resources from fish wastes. However, with an emphasis on sustainability, attention has turned towards nonthermal processing approaches and novel thermal methods that have shown effectiveness in these processes. Despite these advancements, there remains a considerable gap between the lab-proven biorefinery concepts and their industrial implementation for fish waste valorization [76].

By exploring and optimizing intensified bioprocesses, we can unlock the full potential of fish by-products and discards, leading to the creation of valuable products while minimizing environmental impact and ensuring economic feasibility. Bridging the gap between research and industry will be crucial in advancing the sustainable and efficient utilization of fish waste resources.

1.3.1 Challenges and progress made towards achieving “zero discard” fish waste

In alignment with the Sustainable Development Goal (SDG) mandate of achieving "Zero discards" by 2030, many countries, international agencies, and civil societies worldwide are implementing policies aimed at transforming fish waste into value-added resources through sustainable technologies. Achieving this goal requires a careful balance between top-down and bottom-up approaches, considering both cultural attitudes and stakeholder acceptance to ensure successful implementation and widespread adoption of these policies.

Various countries have taken different measures to avoid wastage and promote the effective valorization of fish by-products. Some notable initiatives include:

- FAO Committee on Fisheries: The Food and Agriculture Organization (FAO) has been actively involved in addressing fisheries-related challenges and promoting sustainable practices. The Committee on Fisheries (COFI) plays a crucial role in developing policies and guidelines to reduce wastage and improve the utilization of fish by-products.
- European Union fish discard ban: The European Union (EU) has taken significant steps to reduce fish discards through the implementation of a discard ban. This regulation prohibits the practice of throwing unwanted catch back into the sea, encouraging responsible fishing practices and the utilization of all caught fish.
- Global initiative on food loss prevention and waste reduction: The Save Food Initiative is a global initiative focused on preventing food loss and waste at various stages of the food supply chain, including fish waste. By promoting efficient and sustainable utilization of fish by-products, this initiative contributes to the broader global efforts in achieving SDG targets.

These initiatives, among others, play a vital role in fostering a more sustainable and responsible approach to fish waste management. By engaging stakeholders, adopting sustainable technologies, and implementing well-balanced policies, countries can effectively transform fish waste into valuable resources, contributing to a more sustainable and resource-efficient fishing industry.

It is commendable to see that some countries have taken proactive measures to address the utilization of fish by-products and by-catches. While there may not be strict global benchmarking criteria for resource recovery from fish waste, certain countries have shown leadership by prescribing diversion targets and setting specific goals for fish waste utilization.

Countries such as Austria, Argentina, the Netherlands, Switzerland, Germany, and Norway have implemented policies to promote the efficient utilization of fish waste. For instance, in 2005, Argentina enacted the "Zero fish waste law," which sets ambitious targets for recycling and valorization rates, aiming to reach 80% utilization before 2018. This means that a significant portion of fish waste must be redirected towards beneficial applications rather than being discarded or wasted. Furthermore, the law also prohibits the incineration and landfilling of fish waste unless its energy and bioresource materials are effectively utilized.

By enacting such laws and establishing clear targets, these countries are taking tangible steps towards a more sustainable and responsible management of fish waste. These initiatives not only help reduce the environmental impact of fish waste disposal but also contribute to the development of a circular economy approach, where waste is treated as a valuable resource.

As other nations and regions observe the success and positive outcomes of these initiatives, it may encourage more countries to adopt similar policies and work towards a global standard for resource recovery from fish by-products and by-catches, contributing to a more sustainable and efficient fishing industry.

The Indian aquaculture industry plays a significant role in meeting global demand for fish and seafood products. In this context, the National Fisheries Policy (NFP) framework of 2020 provides strategic guidelines for managing fisheries comprehensively, covering various aspects from capture to pollution control and resource recovery. This policy framework aims to promote sustainable practices and aligns with the objectives set under the Sustainable Development Goals (SDGs).

Additionally, the Indian government has introduced other policies, such as the "Agricultural Export Policy 2018" and the "Blue Growth initiative," which are closely related to the fisheries sector. These policies further support the sustainable growth and development of the fisheries industry in India while also facilitating international trade and export of aquaculture products.

One of the key aspects addressed by the NFP is the modernization of the fish value chain. This entails improving and optimizing the various stages involved in the processing and distribution of fish products. By adopting modern techniques and technologies, the aim is to produce value-added products with extended shelf life, which can enhance the overall competitiveness of Indian aquaculture in the global market.

Moreover, the NFP also emphasizes adherence to international trade regulations. This is crucial for ensuring the quality and safety of Indian aquaculture products exported to international markets. By complying with these regulations, Indian fisheries can gain access to a broader customer base and maintain a positive reputation in the global seafood industry.

Overall, the National Fisheries Policy framework, along with other related policies and initiatives, forms a comprehensive strategy to promote sustainable and responsible fisheries management in India. By embracing these guidelines and fostering a well-regulated fishery sector, India aims to meet global demand for its aquaculture products while contributing to the achievement of the broader Sustainable Development Goals.

1.3.2 Fish waste as a source of value-added products

The terms “fish by-products”, “fish discards”, and “fish side stream” refer to all unused portions of fishes generated during handling and processing operations. Globally, fish processing industries handle approximately 60% of the total catch, amounting to nearly 107 million tonnes, resulting in approximately 27.85 million tonnes of accessible discards [77]. Artisanal fish processors are commonly involved in fish processing, where key processes include drying, fermentation, gutting, deheading, stunning, scaling, meat bone separation, fin cutting, filleting, salting, steaming, and smoking [78]. In traditional fish processing, only about 30-50% of the fish body is utilized, leaving the remaining skin (5%), head (20-25%), and bones (25-35%) unused; these percentages may vary depending on the fish species [79]. Additionally, besides the typical mainstream products of fish bioprocessing industries such as fishmeal, liver oil, fish body, and fish protein concentrates [80], numerous by-products and biowastes are generated, each possessing significant value.

Given the increasing demand for marine-based valuable compounds in the global market, the utilization of fish side streams is gaining recognition as an excellent pathway towards a circular bioeconomy.

The surge in fish consumption has been significant, with fish accounting for approximately 17% of the global population's animal protein intake and 6.7% of all proteins consumed worldwide in 2015 [81]. A diet that includes fish has a profoundly positive nutritional impact and plays a crucial role in addressing unbalanced diets and combating obesity. Fish is not only a rich source of high-quality protein that contains all essential amino acids but also provides essential fats, such as long-chain omega-3 fatty acids, as well as vitamins (D, A, and B) and minerals (calcium, iodine, zinc, iron, and selenium).

These valuable nutritional properties of fish confer numerous health benefits, including protection against cardiovascular disease and support for fetal and infant brain and nervous system development [82]. Therefore, fish consumption contributes significantly to overall health and well-being, making it a valuable component of a balanced and nutritious diet.

Marine species, including fish products, have gained popularity in the medical field and are recognized as a valuable source of animal-derived medicinal products (ADMPs) [83]. Fish byproducts hold great nutritional importance, as they provide proteins, fatty acids, and minerals, with compositions similar to those found in fish fillets and other food products used for direct consumption. Studies focusing on meagre and gilthead sea bream fish species have revealed that the skin is particularly rich in proteins, while trimmings and bones are abundant in calcium, and the head, intestines, and bones offer a good source of lipids [84].

On a dry weight basis, fish byproducts generally contain approximately 49.22–57.92% protein content, 21.79–30.16% ash content, and 7.16–19.10% fat content [85][86][87] The fat content values are slightly higher than those reported in a previous study [84]. Moreover, besides meeting the demand for direct human consumption, the utilization of fish byproducts is gaining increasing attention due to their significant and sustainable reservoir of high-value bio-compounds. These compounds include collagen, peptides, chitin, polyunsaturated fatty acids (PUFAs), enzymes, and minerals, making them suitable for various biotechnological or pharmaceutical applications with considerable market value [88][89][30][90][91][92][93][94][95][96].

In summary, fish byproducts offer a wealth of beneficial components that can be utilized in diverse medical and industrial applications, making them a valuable resource with immense potential for high-value market opportunities.

Indeed, there is a growing demand for specific products in various industries. In the case of mammalian collagen sourcing, which traditionally relies on bovine and porcine bones, fish waste-sourced collagen is gaining attention as a promising alternative. This shift is driven by considerations of technical feasibility, ethical implications, and other factors.

Over the last two decades, the global market for fishmeal (33-69%) and fish oil (55-75%) has experienced significant growth, doubling in size. Moreover, marine-based valuable compounds have consistently maintained higher demand compared to plant-based compounds since 2012 [97]. These trends reflect the increasing interest and recognition of the value derived from marine sources, further reinforcing the potential of fish waste in meeting the rising demands for various valuable products.

Many studies have reported the potential applications of fish waste-based products. For example, fish scales are rich in hydroxyapatite, calcium carbonate, type I collagen, and various minerals and amino acids. Similarly, fishbone has plenty of soluble nutrient components in its tissues, including calcium phosphate and inorganic substances [98]. In addition, it is a rich reserve of organic ingredients such as collagen, gelatine, and other volatile and semi-volatile particles, most of which can find a range of applications in the food and nutraceutical sector [99]. Notably, several compounds hold huge commercial value. For example, the market value for fish gelatine is estimated to upsurge from 412.7 kilotons per year (2015) to 651.7 kilotons (2024), with a market value of US $ 4.08 billion [100].

Numerous studies have thoroughly documented the methodologies employed in extracting these compounds [30][90][101][102][103]. Thus, this section offers an overview of the remarkable potential of fish byproducts, highlighting various applications for the most intriguing compounds derived from these byproducts, such as collagen, peptides, chitin, oil, and enzymes.

1.3.2.1 Collagen and gelatine

Collagen is the predominant structural protein found in the extracellular connective tissues of fish, constituting 15–30% of the total fish protein content [104]. Fish collagen is made up of polypeptide chains composed of repeating triplets of glycine and two other amino acids, mainly proline and 4-hydroxyproline. The utilization of collagen derived from fish skin waste is steadily increasing due to its unique advantages, such as low molecular weight, biocompatibility, minimal antigenicity, high biosafety, and cost-effectiveness. The specific amino acid composition of fish collagen and gelatin has been shown to promote cell adhesion and cartilage production. Notably, fish skin-based collagen has demonstrated exceptional properties for cartilage regeneration in both in vitro (rabbit auricular) and in vivo (rabbit) studies conducted by Sae-leaw & Benjakul (2018) [105], suggesting its potential application in tissue engineering.

Furthermore, research conducted by Zhou et al. (2017) [106] using a mouse model to assess tissue repair mechanisms revealed that collagen derived from Tilapia skin exhibited no cytotoxic effects or adverse inflammatory responses. Gelatin, another biopolymer with significant interest and demand in the food, pharmaceutical, and cosmetic industries, is primarily derived from cows, fisheries, and poultry [107]. In fish, gelatin molecules, composed of a sequence of glycine-proline-alanine, accumulate in bones, cartilage, skin, and other connective tissues. Fish gelatin offers therapeutic advantages, being free from risks associated with spongiform encephalopathy transmission and displaying high antioxidant activity [108].

1.3.2.2 Fish proteins

Fish is indeed an excellent source of proteins, providing well-balanced essential amino acids, including lysine, valine, and phenylalanine. Notably, fish side streams, such as frames and muscles, contain substantial amounts of easily digestible proteins with high bioactivity. These fish proteins exhibit exceptional properties, such as gelling activity, emulsification ability, oil absorption, foaming capacity, and water holding capacity, making them highly valuable for various applications in the food industry [109].

Beyond their nutritional significance, fish proteins offer therapeutic benefits, showcasing immune-modulating properties, antithrombotic effects, and anti-oxidant activities. Studies have shown that proteins extracted from the kidney, fluids, blood, and mucus tissue of fishery by-catch, such as sea bass, display excellent agglutinating and antibacterial activities, and serve as a rich source of bioactive compounds [104]. These bioactive compounds have the potential for various medicinal and pharmaceutical applications, further highlighting the valuable contributions of fish proteins to health and well-being.

1.3.2.3 Fish protein hydrolysates/peptides

Protein hydrolysates and purified peptides are specific protein fragments composed of 2–20 amino acids, obtained through the hydrolysis of fish by-products, such as skin, cod muscles, viscera, and blood. Numerous studies have highlighted fish waste as a valuable source of bioactive peptides, recognizing its sustainable and economic advantages. The functionality of these peptides depends on factors like the type of amino acids, hydrophobicity, and position of the peptides within the protein structure.

Fish collagen peptides, for example, have been found to exhibit ACE inhibitory and anti-hypertensive activities [Caruso et al., 2020]. In recent research focused on radical scavenging activity, fish hydrolysates, including polypeptides extracted from the skin, cartilage, and bone, demonstrated excellent antioxidant properties. Moreover, these peptides showed a protective role against kidney injuries in mice induced with oxidative stress [70]. Specific peptide sequences, such as QGYRPLRGPEFL, derived from skate fish skin, exhibited exceptional levels of β-secretase inhibitory activity, suggesting potential neuroprotective properties [104]. Additionally, fish-based peptides have been shown to enhance mitochondrial activity, restore radical scavengers, and promote gene expression related to elastin production [110].

Beyond the health sector, which significantly benefits from fish waste-based proteins, polypeptides, and hydrolysates, these compounds are also widely employed in the food and nutraceutical industries. Moreover, in the cosmetic industry, protein hydrolysates from fish waste are increasingly used in various formulations aimed at addressing skin wrinkling and improving elasticity. The versatility and beneficial properties of these peptides make them a valuable resource for various industries, contributing to both economic and sustainable practices.

1.3.2.4 Chitin/chitosan

Chitin and chitosan, natural biopolymers derived from marine sources, have a diverse range of applications across various sectors [111]. Chitin is the original biopolymer, while chitosan is the deacetylated form of chitin. The global market demand for chitin was 106.9 thousand metric tons in 2020 and is projected to reach 281.7 thousand metric tons by 2027, with a compound annual growth rate (CAGR) of 14.8% [112]. Chitin extracted from fish by-products alone accounts for an estimated 2000 tons per year [113].

Fish waste-derived chitin finds wide applications in the food and drug industries, mainly due to the highly crystalline nature of the biomolecules. These unique characteristics make fish waste-based chitin particularly suitable for various medical and pharmaceutical applications [111]. For instance, the high degree of deacetylation, net yield, and exceptional stability in adverse environments of fish waste-derived chitosan make it suitable for multiple biomedical applications [114]. These properties of chitosan open up promising possibilities in medical and pharmaceutical research, offering potential benefits for healthcare and drug delivery applications.

1.3.2.5 Enzymes

Enzymes sourced from marine organisms, including fish, play a significant role in the global enzymes market. Particularly, proteases derived from fish are widely used in various commercial applications [115]. Fish proteinases exhibit high activity across different temperature and pH ranges, making them well-suited for several industrial applications. Proteases sourced from fish stomachs are naturally salt-activated, making them suitable for the preparation of fish sauces or fermented products, where certain enzymes from other sources might be inhibited in the presence of sodium chloride [116].

Fish-based enzymes also find applications in unique areas. For instance, pepsin, a heat-labile enzyme extracted from fish by-products, was utilized to produce high-quality caviar, aiding in the quick descaling of Atlantic cod [112]. The activity of fish-based enzymes is often confirmed using spectrophotometry-based enzyme assays [117]. For example, the lysozyme activity in fish mucus tissues was assayed at 450 nm wavelength by adding freeze-dried lysozyme-sensitive bacteria, while alkaline phosphatase activity was assayed at 405 nm by incubating fish skin tissues with p-nitrophenol [118][119]. In both cases, the amount of enzyme released to convert the fish waste substrate into a target product was quantified and compared with reference materials. These assays enable researchers to assess and validate the enzymatic activities of fish-based enzymes, paving the way for their utilization in various industrial applications.

1.3.2.6 Minerals and other valuable compounds with high market demand

Fishery by-products, especially the bones, are a rich source of minerals, constituting approximately 60–70% of the fish-based minerals. These bones contain significant amounts of hydroxyapatite, phosphorus, and calcium [104]. Fish hydroxyapatite, particularly calcium, holds high commercial value and finds various medical and dental applications due to its exceptional thermodynamic stability at human physiological pH. Moreover, these minerals are gaining increasing attention in the food and nutraceutical industries due to their ease of availability, economic advantages, and high biocompatibility [54].

In addition to calcium, fish bones also contain substantial proportions of phosphorus, iodine, magnesium, and selenium, all of which play important functional roles in the human body [100]. In fish waste valorization processes, bones are treated with phosphorus solubilizing microbes and processed to develop biofertilizers, enhancing agricultural productivity [70]. Furthermore, fish-based selenium is highly bioavailable compared to other sources, such as yeast [120]. These valuable minerals derived from fishery by-products offer significant potential for various applications in diverse industries, making them valuable resources for sustainable and beneficial utilization.

Fish waste-based biomaterials and commercially valuable products offer a wide range of applications and nutraceutical value, making them economically attractive under sustainable valorization methods. These products include antimicrobial peptides, anti-freeze proteins, taurine, creatinine, chondroitin sulfate, and astaxanthin. The utilization of marine-based valuable compounds, not only from fish but also from marine microbes, seaweeds, and aquatic plants, is on the rise worldwide.

The demand for these compounds is increasing, with thousands of marine resources being investigated and published annually. Many nutraceutical products with commercial potential are already available in the market. The utilization of fish waste accounts for approximately 25-30% of the whole fish, making it an essential aspect of marine resource utilization.

The major biorefinery products from marine resources include marine oils, functional proteins, and other pharmaceutical and nutraceutical-grade biopolymers. These products hold significant promise for various industries and contribute to the sustainable and economical utilization of marine resources.

1.3.2.7 Fish oil and its applications

Fish oil is a valuable combination of various fatty acids, with a special emphasis on eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The quality of fish lipids stored in subcutaneous tissues, which are typically eliminated as waste, is comparable to the oil obtained from non-waste fractions, ensuring that essential polyunsaturated fatty acids (PUFAs) like EPA and DHA are not lost [115].

The global fish oil market has experienced substantial growth, with a value of $1,905.77 million in 2019, and it is expected to reach $2,844.12 million by 2027, with a compound annual growth rate (CAGR) of 5.79% from 2021 to 2027 [121]. Within the European Union (EU), approximately 120,000 tons of fish oil are produced each year, with Denmark being the largest producing nation. The primary driving force behind this production is the high demand for fish oil as an ingredient in the aquaculture industry, which now consumes as much as 90% of the global fish oil supplies [122].

Despite the significant demand in the aquaculture industry, there is also substantial potential for the high-end and high-value markets, particularly utilizing fish byproducts. As the global trend of fish product processing continues to increase, the volumes of byproducts are also on the rise. In 2016, the global production of fish oil from byproducts accounted for 26% of the total fish oil production [123]. Both the fishing and aquaculture industries generate a considerable amount of oil and fats as a fraction of their finfish processing waste. The amount of oil/fats in the waste depends on various factors, such as the fat content of the specific fish species, the distribution of fat in different fish parts, their age, sex, nutritional status, health, and the time of year [93].

Figure 1.3 Fish oil omega-3 supplements in capsules. (https://www.saga.co.uk/magazine/health-wellbeing/treatments/supplements/omega-3-fish-oils)

For example, it is well-known that the visceral mass of fish discards contains a significant amount of oil or fat, in addition to proteins [124]. As the awareness of sustainability and waste reduction grows, the utilization of fish byproducts, including their oil and fats, offers a valuable opportunity to enhance the value chain and reduce waste in the fish processing industry.

Fish oil is present in various parts of the fish, including the flesh, head, frames, fins, tail, skin, and guts, with varying quantities. Fish typically contains 2–30% fat, and approximately 50% of the body weight is generated as waste during fish processing operations [125]. This waste presents a significant potential for valorization, primarily for human consumption or for the production of biodiesel.

The composition of fish oil mainly comprises triglycerides of fatty acids, which are glycerol combined with three similar or different acid molecules. It may also contain variable amounts of phospholipids, glycerol ethers, and wax esters. Fish oil is highly regarded as the most nutritious and digestible ingredient for farmed fish. The lipid composition in fish is distinct from that of land animals and vegetable oils, mainly due to the significant quantity of long-chain polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid (EPA, C20:5, n-3), and docosahexaenoic acid (DHA, C22:6, n-3), commonly known as omega-3. These essential fatty acids, which cannot be synthesized by the human body, play a crucial role in various critical functions for human health [126][127][128].

Omega-3 PUFAs, particularly EPA and DHA, abundant in fish oil, have well-established usage in nutraceutical and pharmaceutical industries due to their numerous health benefits (Figure 1.3). The consistent quality of fish oil, lower levels of oxidation, and fewer impurities make it an excellent choice for applications that aim to promote health and well-being. Some of the best sources of oily fish rich in EPA and DHA include salmon, herring, mackerel, anchovies, sardines, and tuna [129]. Although fish cannot synthesize omega-3 fatty acids, they obtain them from external sources through their diets, such as algae, microalgae, or plankton.

Besides the health perspective, fish by-products offer opportunities to produce biofuels from triglycerides, presenting an effective alternative energy source. Additionally, recent applications of oil from fish by-products include its use in the leather industry for fat liquoring purposes and as a stimulating agent in bacterial enzyme productions [100]. These versatile applications demonstrate the significant value and potential of fish oil derived from by-products in diverse industries.

Various techniques have been employed to extract oil from whole fish or fish waste, and these techniques have been extensively documented in numerous reviews [93][128][116]. The extraction of fish oil is a crucial process to utilize this valuable resource efficiently and sustainably, benefiting various industries and promoting human health.

The selection of the most suitable method for extracting fish oil depends on various factors, particularly the nature of the waste and the intended application of the oil, with biodiesel production and food supplements being the most common applications [102][130]. Different technologies are applied in the extraction process, ranging from chemical and enzymatic processes to cooking and pressing, as well as more recent green technologies like microwave and supercritical fluids. A comprehensive analysis of different extraction methods applied for the production of omega-3 was conducted by Mendez and Concha [102].

In summary, wet pressing and chemical extraction using solvents are among the most common approaches. However, these methods often involve high pressures and temperatures and result in the residual presence of solvents, which require additional energy-intensive steps for solvent removal. These limitations can restrict their widespread use. To overcome these challenges, less harsh methods that use enzymatic hydrolysis by proteases have been explored. Several enzymes can be employed for this purpose, but studies have shown that alcalases are a more efficient option for the enzymatic extraction process [131][132].

The continuous research and development in this field are aimed at finding more sustainable and eco-friendly extraction methods that can efficiently obtain high-quality fish oil for various applications while minimizing environmental impact and energy consumption. As the demand for fish oil continues to grow, the advancement of extraction technologies will play a vital role in ensuring the efficient and sustainable utilization of fishery by-products and optimizing the production of valuable omega-3 fatty acids for both human consumption and other industrial applications.

Enzymatic hydrolysis offers several advantages as a method for fish oil extraction. It is a quick and easily reproducible process that avoids extreme physical and chemical treatments. Compared to chemical hydrolysis, enzymatic hydrolysis has the advantage of avoiding the generation of chemical waste, making it a more environmentally friendly option. Additionally, it allows for better control of the process and shows great potential for the valorization of fish waste.

Recent research by Araujo et al. demonstrated the efficacy of enzymatic hydrolysis by alcalase in increasing the degree of hydrolysis and oil yield when the initial enzyme concentration was increased [133]. By optimizing the enzyme concentration, they obtained significant amounts of protein hydrolysate, collagen, and oil from fish waste, highlighting the potential of this method for efficient and sustainable fish waste valorization.

However, one drawback of enzymatic hydrolysis is the cost of some enzymes, which can be relatively high, and in many cases, the enzymes cannot be easily recycled. In the context of sustainability, there is a need for more environmentally friendly approaches.

Supercritical fluid extraction (SFE) is an emerging extraction technology that uses solvents, predominantly CO2. It offers several advantages over traditional extraction techniques. SFE utilizes moderate temperatures, reducing the risk of lipid oxidation during the extraction process. It also enables selective extraction of low polar lipid compounds, avoiding co-extraction of polar impurities, such as certain inorganic derivatives with heavy metals [134]. This makes SFE an attractive option for fish oil extraction, especially when seeking a more environmentally friendly and selective extraction process.

As the demand for fish oil and its valuable components, such as omega-3 fatty acids, continues to rise, the development of sustainable and efficient extraction methods becomes crucial. Researchers are exploring various techniques, including enzymatic hydrolysis and supercritical fluid extraction, to optimize fish waste valorization and contribute to a more sustainable and eco-friendly fish oil production.

Indeed, several studies have demonstrated that supercritical fluid extraction (SFE) is a more sustainable and efficient method for extracting oil from fish waste. The application of SFE from the viscera of African catfish (Clarias gariepinus) and common carp (C. carpio) yielded comparable oil extraction efficiency to the traditional Soxhlet method [135][136]. This highlights the potential of SFE as a greener and more environmentally friendly alternative for oil extraction from fish waste.

Additionally, microwave-assisted extraction has been shown to be another cleaner and greener method for oil extraction, particularly for biodiesel production. A study by [137] compared microwave lipid extraction to the conventional solvent lipid extraction (Bligh and Dyer method). They found that the microwave-assisted extraction was approximately 50% more efficient in extracting lipids. Moreover, the study demonstrated that microwave-assisted transesterification, in the presence of KOH catalyst at 65°C for 10 minutes, led to an efficient conversion into biodiesel. Gas Chromatography-Mass Spectrometry (GC/MS) analysis confirmed the presence of essential biodiesel components such as palmitoleic acid, palmitic acid, oleic acid, and eicosapentaenoic acid [137].

These findings emphasize the potential of microwave-assisted extraction as a sustainable and efficient method for obtaining valuable components from fish waste, which can be further utilized in various applications, including biodiesel production. Both SFE and microwave-assisted extraction offer promising avenues for the valorization of fish waste, contributing to more sustainable and eco-friendly practices in the fish processing industry.

The extraction method used can influence the quality of the crude fish oil by potentially introducing impurities [138]. Therefore, a purification process is essential to ensure the fish oil meets the necessary standards for human consumption [139]. Despite its numerous health benefits, fish oil contains highly unsaturated fatty acids that make it susceptible to auto-oxidation. Thus, considering the intended application of the oil, it is crucial to take into account potential oxidation issues right from the initial stages of processing.

Industrial fish processing operations generate substantial amounts of waste, containing valuable long-chain fatty acids that have diverse applications across various markets [116]. These markets include industrial uses, food, feed, aquaculture, and nutraceutical applications (as shown in Table 4). The primary reason for the significant interest in fish oil lies in its rich content of two essential polyunsaturated fatty acids (PUFAs) known as EPA and DHA, also referred to as omega-3 fatty acids.

The applications of these PUFAs are twofold: first, as feed and food supplements, and secondly, in biofuel production. The aquaculture industry is the leading market, demanding oils with low oxidation levels, minimal contaminants, and consistent quality. On the other hand, the nutraceutical market seeks oils with low oxidation and contaminants but high levels of omega-3 fatty acids [141].

The bioactivities of omega-3 fatty acids are well-known and include a range of health benefits. These bioactivities encompass prevention of atherosclerosis, management of arrhythmias, reduced blood pressure, benefits for diabetic patients, protection against manic-depressive illness, relief of asthma symptoms, safeguarding against chronic obstructive pulmonary diseases, alleviating the symptoms of cystic fibrosis, enhancing survival rates of cancer patients, reduction in cardiovascular disease, and improved cognitive function [142][143][144][145]. These extensive health benefits make omega-3 fatty acids highly sought after in various sectors and contribute to the growing interest in fish oil for numerous applications.

Medical research has provided compelling evidence that a diet rich in fish and marine omega-3 fatty acids is associated with a reduced risk of cognitive decline and Alzheimer's disease (AD) [146]. Furthermore, some clinical trials have indicated that supplementation of n–3 PUFA extracted from fish can improve cognitive functioning in elderly adults with mild to no cognitive impairment [147].

Given the well-recognized health benefits of EPA and DHA on human health, researchers have explored alternative ways to provide dietary omega-3 to consumers. One such approach is enriching chicken meat with omega-3 fatty acids derived from sustainable marine sources. A study conducted by Moula Ali investigated this aspect by adding various concentrations of fermentative recovered fish oil (FFO) to broilers' diet. The results showed that EPA and DHA were incorporated into the animals, leading to reduced cholesterol (ranging from 9.2 to 16.6% compared to the control group) and triglyceride levels (ranging from 1.5 to 3.1% compared to the control group) in the serum, liver, and meat of birds fed with FFO. This finding suggests that fish waste-derived oil can indirectly benefit human health through enriched chicken meat [148].

Indeed, various studies have highlighted the antioxidant potential of fish oil. In a study conducted by Sellami et al. [149], oil extracted from the waste liver of three ray species showed a high content of unsaturated fatty acids (UFAs), comprising more than 65% of the total fatty acid content. The prominent n-3 PUFAs present were EPA (C20:5) and DHA (C22:6), with concentrations ranging from 3.36 to 5.51% and from 9.07 to 30.50%, respectively. Interestingly, these oils also contained carotenoids and phenolic compounds, exhibiting antioxidant activity comparable to that of olive oil.

Fish waste-derived oil has shown efficacy at the microbiological level as well, both as a bacterial growth substrate and as an antimicrobial agent. A study by [150] reported the antioxidant and immunomodulatory properties of a combination of fish (salmon) oil with plant extracts, demonstrating a synergistic effect that increased bioactivity compared to fish oil extract alone.

Furthermore, fish waste oil has been utilized to stimulate the production of bacterial enzymes. For instance, microbial lipase production is greatly influenced by medium components such as nitrogen and carbon sources, including fatty acids, triglycerides, and carbohydrates, which can stimulate or repress lipase production. By adding 1.5% cod liver oil to the growth medium of Staphylococcus epidermidis CMST Pi 1, lipase production was significantly increased (14.8 U/mL) compared to other vegetable oils like castor oil and palm oil [151].

The antimicrobial potential of fish oil extracted from S. salar waste samples derived from Italian fish markets was qualitatively characterized and investigated by Inguglia et al. [152]. The researchers utilized GC/MS to analyze the fatty acid composition of the salmon waste oils, which were found to be rich in monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), with a notable presence of omega-3, -6, -7, and -9 fatty acids. The oleaginous extract was then tested against two

Gram-positive bacteria, S. aureus ATCC 6538 and ATCC 25923, and two Gram-negative bacteria, P. aeruginosa ATCC 9027 and ATCC 15442, demonstrating inhibitory effects with a minimum inhibitory concentration (MIC) of 25% and 12.5%, respectively. This inhibition effect was likely attributed to the action of the fish oil on cell membrane alteration and destabilization [153].

Another significant application of fish waste-derived oil is in the production of eco-friendly fuels, particularly biodiesel. Waste oils offer advantages over petroleum and virgin vegetable oil-based fuels, including waste utilization, lower emissions throughout the fuel production life cycle, and a lower price (25 cents per gallon for fish oil compared to $1.19 per gallon for diesel fuel). Additionally, the calorific value of waste oil is similar to that of petroleum distillates [154]. These attributes make fish waste-derived oil a promising and sustainable alternative for fuel production.

Indeed, several studies have explored and confirmed the potential of fish waste oil for biodiesel production. Martins et al. [155] conducted a study on the physicochemical features of fish-based biodiesel obtained from tilapia waste oil, comparing it to the standard requirements established by the Brazilian National Petroleum Agency. The researchers found that the obtained biodiesel met the specifications for specific mass, kinematic viscosity, water content, acidity level, flash point, and oxidation stability, indicating that the residual oil from tilapia waste could serve as a quality raw material for biodiesel production.

Similarly, another study investigated the pyrolysis of waste fish oil, which is an animal source of triglycerides, at 525°C. The results demonstrated that it is possible to obtain biofuels with properties that show good similarity to petroleum-based fuels [156]. These findings highlight the promising potential of fish waste oil as a viable and sustainable source for biodiesel production, offering a more environmentally friendly alternative to conventional fossil fuels.

The potential of fish waste oil has been explored in various applications beyond biodiesel production. Velasquez et al. demonstrated that oil extracted from the viscera of different fish species, including Mexican snook, black seabream, king mackerel, and striped mojarra, can be successfully converted into fatty acid methyl ester through enzymatic catalysis [48]. This process offers a sustainable and efficient way to utilize fish waste oil for various industrial purposes.

In another study, Prakash et al. compared the performance and emissions of Fish Oil Methyl Ester (FOME) with fossil diesel and found that FOME could be a viable alternative fuel for stationary diesel engines [50]. This highlights the potential of fish waste oil as a substitute for conventional fossil fuels in certain applications, contributing to a greener and more sustainable energy landscape.

Moreover, the application of fish waste oil as a fatliquoring agent in leather processing has been investigated. The physical and mechanical properties of leather treated with sulphated fish oil fat, obtained through a sulphation process using sulphuric acid, were found to be superior to those treated with commercial fatliquoring agents [157]. This indicates that fish waste oil-based fatliquoring processes offer environmental safety benefits and cost competitiveness. The techno-economic feasibility of this application was calculated, showing that tanneries could save a significant amount by employing fish waste oil-based fatliquoring processes, making it an attractive option for the industry.

Overall, the research on fish waste oil continues to unveil its potential in diverse sectors, ranging from fuel production to industrial applications and beyond. Its utilization presents a promising avenue for waste valorization and sustainable resource management.

1.3.3 Conventional fish wastage valorization approaches and the need for novel technologies

Indeed, a significant portion of fish waste side-streams is utilized in the production of animal and aquaculture feed. Various unit operations are employed for processing fish waste, such as solvent-solid liquid extraction, wet pressing, smoking, enzymatic hydrolysis, fermentation, and steam distillation, among others [158]. The industrial valorization process often begins with steaming or cooking the waste to prevent enzymatic and microbial spoilage and facilitate the release of water and oil. After centrifugation, the remaining by-products consist of solid cake and press liqueur. Further separation of aqueous, oil, and solid phases can be achieved by heating the liquid portions at around 95°C [116].

To produce fish discard flour, hot air drying is employed until it reaches a moisture content of 10% or less to prevent microbial growth. However, this drying process may cause degradation of thermolabile volatile and semi-volatile compounds [159].

In the USA, companies like Gurry Investments, Multi Bloom, and Mega Green utilize hydrolysis processes to convert fish offal waste obtained after filleting into high-quality fish oil and organic fertilizers, reporting higher profits compared to fishmeal [160]. Similarly, Tidal Vision, another US-based company, focuses on value addition of fish skin by employing a unique tanning process to produce wallets and extracting chitin and chitosan from fish waste and by-catch using an eco-friendly method, finding various applications in medicine and agriculture [161].

Fish by-products are rich sources of fats, proteins, and various micronutrients that are valuable for industries such as cosmetics, biomedicine, and pharmaceuticals. However, the resources in fish waste biomass are distributed heterogeneously, demanding precise control of bioconversion technologies to fully harness their potential. Proper utilization of these valuable resources from fish waste can not only reduce waste but also contribute to sustainable and economically viable practices.

Fish waste, being highly perishable and with a high microbial load, poses significant challenges during processing. The organic portion of fish waste decomposes rapidly due to autolytic damages, oxidation, microbial spoilage, and enzymatic lysis of fleshy fish tissues [104]. Conventional processing methods often involve high temperatures above 150°C to achieve safe moisture content and inactivate enzymes. However, this can lead to the degradation of valuable heat-sensitive compounds.

The high lipid and protein content of fish by-products makes them prone to oxidation during processing, resulting in the accumulation of off-flavors that alter their organoleptic properties and reduce their acceptability [70]. To produce fish protein hydrolysates, the enzymatic hydrolysis process is employed, followed by the removal of bones [162]. Bioactive peptides from fish discards are prepared through hydrolysis and fragmentation of proteins extracted from the waste biomass [163].

Anaerobic digestion and co-digestion of fish waste streams with vegetable waste offer potential for biomethane production [164]. The biogas produced can be used for preserving fish through smoking and drying. However, their impact on nutritional value, safety, and overall sensorial acceptability remains a concern. Hydrothermal carbonization produces hydrochar from liquid fish waste biomass, but the use of "high-value" biomaterials in this process is still uncertain [165]. The challenges in processing fish waste underscore the importance of developing sustainable and efficient valorization methods to fully utilize the valuable components and minimize waste.

Indeed, the valorization of fish waste presents various challenges, especially in maximizing nutrient bio-absorption and obtaining high-quality products. While adding nutrient-dense fish discards to the diet seems simple, enhancing bioavailability and bioactivities requires extraction, purification, and concentration of valuable compounds [166]. Polymers like chitin and chitosan can be obtained from fish waste after undergoing deproteinization, demineralization, and deacetylation processes [167].

The extraction approaches used to recover valuable compounds can significantly impact the organoleptic quality and functional potential of the end-products. Various factors such as fish species, matrix composition, pH of the medium, process temperature, and extraction time influence the final outcome (Pedro et al., 2019). Challenges related to scalability, energy consumption, extraction costs, time requirements, analytical reproducibility, and product quality need to be addressed [168].

For instance, the wet reduction process is effective in extracting oil from fish scales but involves high temperatures that may lead to degradation of essential fatty acids due to oxidation reactions and hydrolysis [102] Fish skin is rich in antibacterial proteins, lectin, immunoglobulins, and more, but the mucus covering the skin can complicate conventional extraction processes, limiting the recovery of valuable biomolecules [70]. The degree of hydrolysis during fish protein extraction can also result in the production of unstable peptides with variable lipid peroxidation, further emphasizing the need for a precise and tunable valorization process to achieve desired product quality [169]. Overall, addressing these challenges is crucial to fully utilize the potential of fish waste in producing high-quality and valuable products.

Fish waste valorization methods vary depending on the economic advantages and technological capabilities of different countries. Some common methods include sun-drying, salting, smoke-drying, and solvent extraction, with developing countries often utilizing solar driers to produce better quality dehydrated fish products. Fishmeal and fish oil are routinely produced through wet pressing and hydrophobic solvent extraction, respectively, while biofuels are obtained through esterification processes [170]. Notably, more than 35% of fishmeal and fish oil currently come from fish by-products in developing countries [171].

In developed countries like France, Japan, and Southeast Asia, enzyme technologies are commonly used to produce protein hydrolysates on a large scale, though their adoption is limited in other countries due to cost factors. Some countries, such as Barbados and Saint Kitts & Nevis, have strengthened their fish supply chain strategies to maximize the potential of fish waste products, while others like Bangladesh and Thailand have optimized fish silage processing through chemical acidification technology [172].

However, despite the progress made by developing countries in utilizing fish waste materials, many countries still lack interest in processing them to extract valuable components due to economic reasons and technological gaps [173]. Conventional processes for fish waste valorization are often energy and resource-inefficient and rely on the use of several chemicals. Therefore, there is a need to create effective strategies to utilize fish by-products for producing commercially important value-added products, leveraging technological advancements to address gaps in the current knowledge on fish waste valorization.

Countries worldwide are making efforts to frame policies and promote technological advancements in fish waste valorization to align with the Sustainable Development Goals (SDGs) and achieve the objective of "zero discard" of fish waste. The growing health and sustainability awareness among millennials has further highlighted the importance of the biorefinery concept in this context. As a result, there has been a significant focus on research aimed at creating value-added nutraceutical compounds from fish waste.

The concept of bio-intensification in fish waste valorization has gained traction, offering the potential to recover even more valuable compounds from the waste. The exploration of these bio-intensified strategies holds tremendous promise for enhancing the overall value derived from fish waste. However, despite the vast potential of these approaches, it is crucial to move biorefinery technologies from laboratory research to practical industrial applications.

By bridging the gap between research and industrial implementation, the utilization of fish waste can be optimized, benefiting both the environment and the economy. Emphasizing the biorefinery approach not only addresses the challenge of fish waste disposal but also opens up new avenues for sustainable and value-driven solutions, contributing to the broader goal of achieving a more sustainable and circular economy. Continued research and collaboration between academia, industry, and policymakers will play a crucial role in realizing the full potential of fish waste valorization and its positive impact on society and the environment.

1.3.4 Shelf-life determination of fish waste-based compounds

The storage stability of fish waste-based valuable products is essential to ensure their optimal sensory and chemical qualities during storage and consumption. The key parameters that determine the shelf-life of these products are water activity (aw) and microbial load. However, other intrinsic and extrinsic factors also play a role in quality changes during the storage period.

External factors that can impact the characteristics of fish waste-based products include the level of oxygen exposure, intensity of light, relative humidity in the storage environment, and the conditions of packaging. These factors can influence the oxidation and degradation of the products, leading to changes in their sensory attributes and chemical composition.

On the other hand, intrinsic factors like surface hydrophobicity, the formation of reducing sugars, and changes in pH can also contribute to the deterioration of the products during storage. These factors can affect the stability of proteins, lipids, and other bioactive compounds present in the fish waste-based products.

To ensure the best storage stability, it is crucial to implement proper storage conditions and packaging techniques that can protect the products from external factors such as oxygen and light exposure. Additionally, monitoring and controlling intrinsic factors like pH and sugar formation can also help maintain the quality of fish waste-based products over time. Regular quality assessments and testing are necessary to ensure that these products meet the required standards and remain safe and beneficial for consumption.

The shelf-life of fish waste products with a protein matrix is typically analyzed based on the degree of hydrolysis and glass transition temperature. However, such products may undergo undesirable physical changes during storage, such as caking, stickiness, and structural collapse due to the presence of liquid bridges between protein molecules. These changes can be influenced by factors like storage temperature and water activity (aw). For instance, myofibrillar protein hydrolysates from tilapia fish waste showed increased hardness at certain aw levels and peptide aggregation through Maillard reactions at other aw levels during a 4-month storage study [174].

In the case of amino polysaccharides like chitin extracted from fish waste, the degree of acetylation, solubility, and chelating capacity are generally assessed to evaluate its quality deterioration over time during storage [175]

For oily fish products, the evaluation of shelf-life mainly focuses on oxidative and hydrolytic spoilage. Due to the high content of polyunsaturated fatty acids in fish waste-based oils, they are prone to rapid oxidation, leading to deterioration. Parameters like acid, peroxide, anisidine, thiobarbituric acid, and unsaponifiable matter are commonly used to assess the quality of fish oil [176]. A shelf-life study on fish oil encapsulated with fish gelatin and ginger oil demonstrated that the encapsulated product exhibited lower lipid oxidation compared to the control during a 25-day storage period under refrigeration [177].

In summary, the shelf-life of fish waste products is determined by assessing various parameters related to their composition, structure, and quality changes during storage, taking into account factors like water activity, temperature, and oxidative stability. Regular monitoring and evaluation of these parameters are essential to ensure the quality and safety of these valuable products for various applications.

1.4 Omega-3 fatty acids

A fatty acid is a carboxylic acid with a long aliphatic chain that can be either saturated or unsaturated, particularly in the fields of chemistry and biochemistry [178]. Naturally occurring fatty acids typically consist of an even number of carbon atoms, ranging from 4 to 28. However, they are not found in their free form in certain species, such as microalgae, but rather exist as three types of esters: triglycerides, phospholipids, and cholesteryl esters [179].

Fatty acids play a crucial role in nutrition, serving as a significant source of fuel for animals and acting as structural components of cells in the various forms mentioned earlier [180]. Since the 1970s, researchers have been intrigued by the low occurrence of cardiovascular disease among Eskimos living in Greenland, leading to the investigation of omega-3 fatty acids [181].

Studies from different countries have revealed that omega-3 fatty acids offer numerous health benefits, including anti-inflammatory, anti-thrombotic, anti-arrhythmic, blood lipid level reduction, and vasodilatory properties [182]. The research on omega-3 fatty acids has led to the recognition of their importance in maintaining cardiovascular health and overall well-being.

The Food and Agriculture Organization of the United Nations' (FAO) recommendation on food fats and oils in October

1993 highlights the essential role of omega-3 and omega-6 fatty acids in human nutrition. Fatty acids play a crucial part in forming cell membranes and are also precursors to eicosanoids, such as arachidonic acids, which are biologically active compounds involved in various physiological processes [183].

According to the FAO's recommendation, maintaining a balanced ratio of omega-6 to omega-3 fatty acids in the diet is important, ideally at a ratio of 5:1 or 10:1. If this ratio is disrupted, individuals are encouraged to consume more omega-3-rich foods, such as green leafy vegetables, legumes, fish, and other seafood [184]. Ensuring adequate consumption of essential fatty acids is particularly important during pregnancy and breastfeeding to support the development needs of the fetus and child [185].

By following these recommendations and incorporating a balanced intake of omega-3 and omega-6 fatty acids, individuals can support their overall health and well-being while meeting the specific developmental needs of pregnant and breastfeeding individuals and their children.

Omega-3 fatty acids are essential long-chain polyunsaturated fatty acids (PUFAs) present in both plants and marine organisms. The main types of omega-3 fatty acids are docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and alpha-linolenic acid (ALA) [186]. Alpha-linolenic acid (ALA) is a plant-based essential omega-3 polyunsaturated fatty acid that contains three double bonds [187].

Perilla oil contains the highest percentage of α-linolenic acid at 67%, followed by linseed oil at 55%, peony oil at 42%, sea buckthorn oil at 32%, Bama hemp oil at 20%, rapeseed oil at 10%, soybean oil at 8%, and grape oil at 50% [188]. Currently, perilla seed oil is recognized as the edible oil with the highest α-linolenic acid content. The second and third sources of omega-3 fatty acids are derived from animals and fish, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which contain five and six unsaturated bonds, respectively [189].

The varying sources of omega-3 fatty acids provide different dietary options for individuals seeking to incorporate these essential nutrients into their diets, each with its unique health benefits.

Polyunsaturated fatty acids (PUFAs) give rise to a diverse array of biologically active compounds, and these compounds play crucial roles in both pathological and physiological processes. It is essential to understand the contributions of these active compounds, particularly in the context of the increasing prevalence of inflammatory diseases that can result from imbalances in the body's PUFA levels [190].

The Western diet, characterized by an increased ω-6:ω-3 PUFA ratio, has been associated with an enhancement of inflammatory processes, which can predispose or exacerbate various inflammatory diseases [191]. This imbalance in the PUFA ratio leads to alterations in the production of important mediators and regulators of inflammation and immune responses, favoring a pro-inflammatory profile. It is crucial to recognize that an unbalanced dietary intake of ω-6:ω-3 PUFAs can have detrimental effects on human health. Therefore, investigating the potential therapeutic effects of dietary supplements containing ω-3 PUFAs for alleviating inflammatory diseases is of significant importance [192].

Understanding the impact of different PUFA ratios on inflammatory processes can inform dietary interventions and nutritional strategies aimed at promoting better health and mitigating inflammatory conditions. Further research into the specific roles and effects of PUFA-derived active compounds is necessary to develop targeted approaches for managing inflammatory diseases effectively.

1.4.1 Literature review

Numerous studies have highlighted the positive health effects of both saturated fatty acids and polyunsaturated fatty acids (PUFAs), particularly omega-3 fatty acids (ω-3). Omega-3 fatty acids have been associated with various health benefits.

Research data suggests that a decrease in omega-3 fatty acid concentration may be linked to mood disorders [193]. Preliminary findings indicate that omega-3 fatty acids could potentially be effective in treating mental disorders such as bipolar disorder, schizophrenia, and dementia, making it a safe and beneficial treatment option for pregnant and nursing women [194].

Moreover, numerous studies have demonstrated that consuming foods rich in long-chain omega-3 fatty acids, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), such as fatty fish, is associated with a reduced risk of coronary heart disease [195]. For pregnant women, essential fatty acids are critically important for the growth and development of fetuses and infants, particularly in brain and vision development. A well-balanced diet during pregnancy results in the deposition of approximately 2.2 grams of essential fatty acids daily in both maternal and fetal tissues, emphasizing the importance of omega-3 intake during pregnancy [196].

Overall, the evidence suggests that both saturated and polyunsaturated fatty acids, especially omega-3 fatty acids, play significant roles in supporting various aspects of human health, including mental health, cardiovascular health, and fetal development. Including sources of omega-3 fatty acids in the diet is vital for promoting overall well-being.

In 2004, the US National Food and Drug Administration (FDA) made an announcement stating that omega-3 fatty acids are a quality healthy food that can help reduce the risk of coronary heart disease [197]. Omega-3 fatty acids are vital nutrients for human health, but the human body cannot synthesize them on its own; they must be obtained from dietary sources.

Dr. Jing X. Kang, the director of the Harvard Center for Fatty Acid Research, has emphasized the significance of fatty acids, along with vitamins and amino acids, as essential nutrients for the human body. Among fatty acids, omega-3 and omega-6 are considered essential because the human body cannot synthesize them and must obtain them through the diet [198].

The importance of incorporating sources of omega-3 fatty acids into the diet cannot be overstated, as they are crucial for various aspects of human health, including cardiovascular health, brain function, and immune system regulation. Consuming foods rich in omega-3 fatty acids, such as fatty fish, flaxseeds, and walnuts, is essential for maintaining optimal health and well-being.

Research has indeed shown a significant link between an imbalance of omega-3 (ω-3) in the diet and impaired brain function and cognitive diseases. Omega-3 fatty acids play a crucial role in the structure of brain cells, as they are the main components of the brain's cell membranes. Incorporating omega-3 fats into the cell membrane increases its fluidity, which is essential for maintaining normal brain cell function. Higher membrane fluidity facilitates the brain's ability to adapt to new information and process it effectively. Omega-3 fatty acids integrated into the cell membrane also contribute to the proper functioning of neurotransmitter receptors, promoting efficient information transmission within the brain. Neurotransmitters are essential chemicals in the brain that transmit information between brain cells and the body.

Preliminary studies have shown that omega-3 fatty acids can stimulate the secretion of brain growth factors, which in turn contributes to the synthesis of brain messengers and reduces the degradation of these messengers (Wu et al., 2004). This supports healthy brain function and may have positive effects on cognitive processes and mental health.

Numerous epidemiological studies have established the importance of omega-3 fatty acids for overall human health. Enriching the diet with omega-3 has been associated with the prevention of various diseases, such as cardiovascular diseases, myocardial infarction, psoriasis, bowel diseases, mental diseases, certain types of cancer, and bronchial asthma [199]. The evidence supports the notion that including sufficient omega-3 fatty acids in the diet can have a protective effect on overall health and contribute to the prevention of various diseases.

Omega-3 fatty acids are classified as essential since they are crucial for maintaining good health, but our bodies cannot produce them, necessitating their intake through food sources. These fatty acids play a vital role in supporting brain function and act as precursors to anti-inflammatory hormones, thereby helping to reduce inflammation within the brain [200].

Both ω-6 and ω-3 polyunsaturated fatty acids (PUFAs) are essential nutrients that must be acquired through the diet, as humans and other mammals lack the necessary endogenous enzymes for ω-3 desaturation.

When both ω-3 and ω-6 are present in the diet, they compete with each other during the conversion process to their respective final products. As a result, the ω-6:ω-3 ratio directly impacts the types of eicosanoids produced. This competition is particularly significant because the discovery of thromboxane as a platelet accumulation factor highlights its role in thrombosis [201].

Moreover, leukotrienes, which are highly active lipid compounds formed in the body from arachidonic acid with a 20-carbon chain, play a critical role in the immune and inflammatory systems. Consequently, they are linked to conditions such as arthritis, lupus, and asthma [202].

The discovery of the role of ω-6 eicosanoids has sparked significant interest in finding ways to regulate their synthesis. One effective approach is to increase the consumption of ω-3 fatty acids while reducing the intake of ω-6 fatty acids. Notably, eicosapentaenoic acid (EPA), an ω-3 fatty acid, gives rise to potent anti-inflammatory nano molecules known as resolvins within the body. Furthermore, research has revealed that ω-3 also converts into other anti-inflammatory molecules called omega-3-oxylipins [203].

Overall, the favorable health effects associated with ω-3 fatty acids are attributed to their ability to inhibit or modulate eicosanoid pathways, leading to altered inflammatory responses and changes in the activity and expression of associated proteins and enzymes involved in various signaling pathways for normal and pathological cell function. Additionally, including ω-3 fatty acids in membrane phospholipids and their direct impact on gene expression contribute to their positive effects [204].

Given the interconnected nature of these pathways, the biological potential of ω-3 fatty acids in promoting health and managing disease likely results from a combination of coordinated mechanisms. Consequently, ω-3 fatty acids can be considered highly functional food components with numerous beneficial properties.

An imbalanced ratio of ω-6 to ω-3 polyunsaturated fatty acids (PUFAs) in favor of ω-6 PUFA is associated with a prothrombotic and proinflammatory environment, contributing to the development of conditions like atherosclerosis, obesity, and diabetes. Conversely, populations with regular diets rich in ω-3 PUFA, such as Icelandic, Inuit, and Native American communities in Alaska, have shown lower incidences of these diseases. However, it is essential to note that using fish oil as the primary source of omega-3 PUFA for treating type 2 diabetes has not always yielded consistent success. Blood levels of ω-3 may vary depending on factors like lifestyle (e.g., fish intake), geographical location, and genetic influences [205].

Early epidemiological studies have demonstrated that high levels of ω-6 fats may exacerbate cardiovascular risk by promoting inflammation, while the intake of ω-3 PUFA, conversely, exerts anti-inflammatory, antioxidant, and metabolic effects. Increased consumption of ω-3 PUFA has been associated with a reduced risk of cardiovascular disease.

According to Narinder Kaur from Punjab Agricultural University, ω-3 fatty acids offer potential benefits for heart health and may also have positive effects on conditions like cancer, diabetes, and neurological disorders. The anti-inflammatory properties of ω-3 fatty acids make them useful in treating inflammatory diseases, and specific population groups, such as pregnant/nursing women, infants, and children, may also experience advantages from sufficient ω-3 fatty acid intake.

Maintaining a balanced ratio of omega-6 to omega-3 is crucial, as an elevated ratio of omega-6 to omega-3 can lead to a pro-inflammatory state in the body, including brain inflammation [201]. Since omega-3 and omega-6 compete in the cell membrane, achieving the proper balance between these two substances is essential to promote an anti-inflammatory environment.

It is important to note that while ω-3 and ω-6 fatty acids have different effects on the human body, strong evidence supports the necessity of maintaining a balanced and proportional consumption of both essential fatty acids for overall heart health and general physical and mental well-being [206]. According to the American Dietary Association, adults should obtain 20-35% of their energy from dietary fats while avoiding "harmful" saturated and trans fats. Emphasizing ω-3 fatty acid intake is also recommended.

The typical American diet often contains omega-6 fatty acids at levels 14-25 times higher than that of ω-3 fatty acids, leading to an imbalanced ratio. In contrast, the Mediterranean diet promotes a healthier equilibrium between ω-6 and ω-3 fatty acids. Research has indicated that individuals following a Mediterranean diet are less susceptible to heart disease. This diet is characterized by low meat consumption, as meat tends to contain higher levels of ω-6 fatty acids. However, beef from grass-fed sources tends to have a more favorable ratio of ω-3 to ω-6 fatty acids.

To maintain overall health, it is important to achieve a balanced ratio of ω-6 to ω-3 fatty acids. The recommended ratio should ideally be between 2:1 and 4:1, with some health experts advocating even lower ratios. Studies have shown that a ratio of 2-3:1 between ω-6 and ω-3 fatty acids can help reduce inflammation in patients with rheumatoid arthritis, while a ratio of 5:1 has beneficial effects for asthma patients. Conversely, a ratio of 10:1 has a negative effect. For patients with colorectal cancer, a ratio of 2.5:1 has been found to reduce the proliferation of rectal cells, whereas a ratio of 4:1 has no effect [207].

Maintaining an appropriate balance between ω-6 and ω-3 fatty acids is crucial for promoting overall well-being and may have significant implications for preventing and managing various health conditions.

The past few decades have seen significant changes in nutrition, with a notable increase in fatty acid intake. Industrialized societies now demonstrate a higher consumption of saturated fats, ω-6 PUFA, and trans-fatty acids, while the intake of omega-3 PUFA has decreased overall. Fatty acids currently constitute 28-42% of the total energy consumed by the European population, a rise compared to ancestral nutrition where fatty acid intake was only around 20-30% of total energy [208].

Due to the increased use of vegetable oils rich in ω-6 PUFA in the Western diet, the consumption of ω-6 PUFA has far exceeded that of ω-3 PUFA. Ideally, the dietary ω-6:ω-3 ratio should be around 1-4:1. However, the Western diet has led to an alarming increase in this ratio, reaching as high as 10:1 up to 20:1 [208].

Studies have demonstrated that the most beneficial ratio for health is closer to 1:1 between ω-6 and ω-3 in the modern diet, but the current ratio often reaches 15:1. Achieving a healthier balance can be accomplished by consuming more foods rich in ω-3, such as cold-water fish, taking appropriate supplements, and reducing the consumption of processed foods and vegetable oils.

As the awareness of the importance of ω-6 and ω-3 fatty acids in our diet grows, an increasing number of clinical and experimental studies are being conducted to support nutritional guidelines for these components. Maintaining an appropriate ratio of ω-3 and ω-6 fatty acids is crucial not only for normal growth and development but also for the prevention and treatment of certain diseases.

The ongoing research and scientific evidence related to ω-3 and ω-6 fatty acids can play a pivotal role in helping populations achieve optimal health by encouraging dietary adjustments to improve the balance of these essential fatty acids.

1.4.2 Omega-3 ingredient market

Fish are the primary commercial source of omega-3 fatty acids, with their oil containing approximately 20%-30% DHA. According to the Global Organization for EPA and DHA Omega-3 (GOED), the market for omega-3 ingredients, including EPA and DHA, reached a value of US$ 1.53 billion in 2021, representing a 5.5% increase compared to the previous year. The global omega-3 market's volume also rose by 2.1% year over year, reaching 115,031 metric tons ("2022 Global EPA & DHA Omega-3 Ingredient Market Report 2020–2021 Data & Forecasts Through 2024," 2022).

Omega-3 fatty acids find applications as ingredients in various consumer products, such as dietary supplements, infant formula, clinical nutrition, fortified food products, and pharmaceuticals. In 2021, the market for finished EPA and DHA omega-3 products was valued at US$ 47 billion, with an expected average annual growth rate of 3.2% between 2022 and 2023, especially in regions like Asia, Africa, and the Middle East ("2022 Global EPA & DHA Finished Products Report: Data for 2020 and 2021 with Forecasts through 2023," 2022).

The projected future growth for the omega-3 portion of the worldwide essential fatty acids market is significant. By the end of 2032, it is expected to surpass US$ 3.3 billion, with an estimated annual growth rate of 10.0%. The market volume is also anticipated to increase substantially from approximately 160,021 metric tons in

2022 to 417,518 metric tons by the end of 2027 ("Essential Fatty Acids Market Outlook (2022–2032)," 2022).

1.5 Fish/fish waste-based fertilizers

The processes involved in raising, catching, and processing fish generate significant amounts of waste, which has become a global concern [209]. For each tonne of fish consumed, approximately an equal amount of fish waste (FW) is discarded through ocean dumping or land disposal. The term "fish waste" encompasses various materials, such as whole fish (dead or damaged fish), fish trimmings, and specific tissues, including heads, intestines, tails and fins, skins, scales, and bones, among others.

Significant quantities of fish waste (FW) are generated in the primary capture fisheries nations, including Canada, the USA, India, the Republic of Korea, China, Spain, and Norway [210]. Depending on the conversion process, FW can make up about 30–45% of the original weight of the fish product in fish markets or fish processing industries. Due to fish processing and underutilization, FW is likely to account for approximately 30 – 70% of the initial fish weight [211].

The utilization and further processing of fish waste (FW) depend on local conditions and the structure of the industry. FW can be processed into proteins, amino acids, peptides, collagen, oil, minerals, enzymes, flavors, and other compounds for food, feed, technical, and pharmaceutical purposes. In cases where FW doesn't meet the relevant standards for food or feed, it can be employed for energy production or as fertilizers [211]

The utilization of FW for fertilizer production (Figure 1.4) has garnered attention over the years due to its potential to enhance the economic and ecological sustainability of the fish industry [211]. The practice of using fish and shellfish as fertilizers dates back to ancient times. In medieval France, shellfish debris was employed along the coast to enrich crop growth [212]. Similarly, Egyptians, Incas, and Mayans used fish as fertilizer. In coastal areas, fish residues were traditionally utilized to fertilize crops. For example, in Nordland County, Norway, the backbones and heads of cod, as well as leftover herring, were used directly or composted to fertilize both leys and row crops [212]. Around the 1880s, there was a growing interest in commercial fertilizers, which led to the establishment of several factories producing "fish guano" in Norway. These factories processed heads of cod and other fish waste (FW) through steaming or treatment with sulfuric acid. The resulting product was then dried, ground, and exported, including to countries like Germany [212].

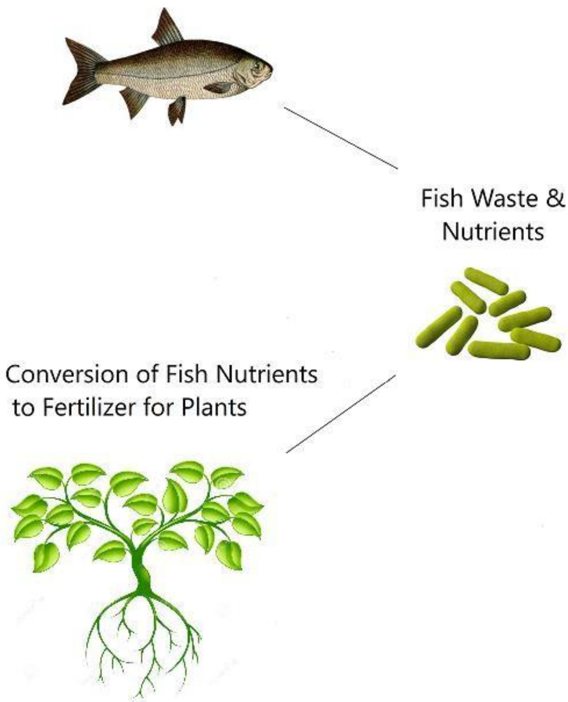

Figure 1.4 Fish waste as fertilizer (adapted from https://www.lifeforceorganics.us/aquaponics).

Currently, commercial fish-based fertilizers are available for both agricultural and horticultural crops, but they are not commonly used in Northern Europe, especially in organic agriculture, where one might expect a relatively higher interest in such fertilizers. These fish-based fertilizers come in various formulations, including meal, bone meal, liquid fertilizer, and compost, and they may also be blended with seaweeds. As of January 25, 2020, the Organic Materials Review Institute (OMRI), an international non-profit organization that determines which contributions are permitted for use in organic production and processing, had listed 157 fish-based fertilizers approved for use in organic agriculture, demonstrating the profitability of this industry [213]. The wide availability of commercial fish-fertilizer products indicates that this industry is economically viable.

Fish meal has been approved as a fertilizer in organic farming since the first public regulations were published by the EU in 1991 [214]. In organic farming, many fertilizers are derived from various types of organic materials, such as by-products, household waste, or food industry sludge. Due to this organic origin, different regulations apply to the content of environmental toxins, pathogens, plastics, etc., in addition to the organic regulations. An organic fertilizer must declare its nutrient content, specifying elements such as nitrogen (N), phosphorus (P), and potassium (K) using their chemical symbols in the order N-P-K, or magnesium (Mg), calcium (Ca), sulfur (S), or sodium (Na) using their chemical symbols in the order Mg-Ca-S-Na [215].

Organic farming systems are built on the principle of nutrient recycling through organic inputs, aiming to sustain the fertility of soil-plant systems while preserving a balance between food production and environmental preservation. Phosphorus (P) has been recognized as a crucial raw material for the EU [216], indicating the need for the development of new management strategies for this essential plant nutrient mineral and its role in the food system. Together with other nutrients, P is lost from agricultural soil or intentionally transported into the sea as part of societal infrastructure, including sewage systems. Consequently, using sea-derived fertilizers on field-grown crops involves the recycling of terrestrial nutrients. Sustainable harvesting of natural resources aligns well with the principles of organic farming, particularly the fundamental principle of ecology. According to the International Federation of Organic Agriculture Movements (IFOAM), the principle of ecology emphasizes that Organic Agriculture should be based on living ecological systems and cycles, work with them, emulate them, and help sustain them [214].

Recycling of nutrients is an integrated part of the increasingly popular circular economy, for instance in the EU, which aims to limit or avoid the production of waste through reutilization of secondary raw materials. The EU action plan for the circular (Blue) economy includes policies for waste management, such as legislative proposals on waste and landfills, proposed changes to extended produce, remanufacture, or recycling [217]. This policy fits well with better utilization of residual organic materials, for instance, as fertilizers. In this context, fish products have relevance since they can recycle P, a scarce resource, from the sea and back to terrestrial environments. Reducing waste and pollution by recycling and through efficient exploitation of renewable resources is the general interest of organic farming and agrees very well with the concept of a circular and bioeconomy [212].

1.5.1 Nutrients in fish and FWs suitable as crop fertilizers

Crop plants typically contain around 30 g of minerals per kg of fresh plant material, with six macronutrients, namely nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S), forming a significant part of this amount. While fertile soils can provide a substantial portion of these nutrients, many soils rely on regular fertilization to supply all macronutrients and preferably thirteen micronutrients required for successful crop growth.

Fish-based fertilizers are known to contain significant amounts of N, P, and Ca compared to the demands of crop plants for these nutrients. Additionally, these fertilizers usually contain S, but they may not be as well balanced concerning the needs of crop plants for the macronutrients K and Mg. The importance of N as one of the primary elements for crop production is evident from the billion-dollar mineral N fertilizer industry. Similarly, P is another crucial macronutrient essential for agriculture and food production. Over 85% of the applied P in agriculture comes from phosphate rock, which is a limited resource [218]. This highlights the need for sustainable nutrient management strategies, such as using fish-based fertilizers, to address the demand for these essential nutrients while considering the limitations of traditional phosphate rock sources.

Soil characteristics play a crucial role in the availability of phosphorus (P) to crop plants. For instance, Lateritic soil, commonly found in tropical regions, has a very high binding capacity for phosphate, which restricts P availability to plants. Similarly, calcareous and alkaline soils with high pH levels also limit the plant availability of P. Consequently, fertilizers rich in P can be valuable in addressing these limitations.

Fish bones consist of 60-70% minerals, primarily Ca and P in the form of hydroxyapatite (Ca10(PO4)6(OH)2) (Ghaly et al., 2013; Kim and Mendis, 2006). Fish scales are also nutrient-rich, particularly in nitrogen (N), P, and Ca. They are composed of a layer of hydroxyapatite and calcium carbonate surrounding a kernel of collagen, which is a protein [212].

In a study focused on describing the risk of accumulating radioactive minerals in seafood [212], elemental composition analysis of tissues from various pelagic fish species revealed that Ca, P, K, Mg, sodium (Na), silicon (Si), and aluminum (Al) were the most common elements found in different organs. However, N contents were not analyzed in that study.

The nutritional composition of fish can vary depending on several factors, including the type of capture (inland or marine), fish size, and the inclusion or exclusion of specific tissues such as head, bones, and viscera.

According to the study by Bogard et al. (2015), the average values of N-P-K for fish from inland capture were 120:11:13, while those from marine capture were 130:16:11. Additionally, the average contents of Ca in fish from marine capture were observed to be 21.0 g per kg dry matter (DM).

Palani et al. (2014) reported that the average Ca contents in edible fish parts were 31 g per kg DM. The average N contents were similar in both studies, ranging from 12% to 13% of DM.

In a study of cod head bones after pilot-scale hydrolysis to remove muscle tissue (Ahuja and Løes, 2019), high concentrations of Ca (300 g per kg DM) and P (120 g per kg DM) were confirmed.

Another study by Toppe et al. (2007) investigated bones from various fish species following different processing steps, with subsequent freeze-drying. The N contents in fish bones ranged from 4% to 7% of DM, P contents from 90 to 123 g per kg DM, Ca contents from 140 to 239 g per kg DM, and Mg contents from about 2 to 4 g per kg DM [212].

Fish powder obtained from the processing of dried tuna frames and gills (Abbey et al., 2017) showed high calcium contents compared to powder from trimmings. The calcium contents were reported to be 144 g per kg DM for frames and 166 g per kg DM for gills, compared to 11.2 g per kg DM for trimmings.

Indeed, the analysis of fish and fish waste (FW) from various studies reveals substantial amounts of minerals, particularly phosphorus (P), calcium (Ca), and nitrogen (N), which could be valuable in meeting the nutritional requirements of crop plants.

Regarding P content, the powder obtained from frames and gills showed close to twice as high P content compared to powder from trimmings. A study comparing incinerated waste bones from fish and chicken for struvite precipitation found that fish bones had somewhat higher concentrations of P and Ca compared to chicken bones [219]

The N content in fish scales from different fish species varied from about 5% to 11%. A recent study analyzed P and Ca contents from processed by-products, such as fish bones, gills, guts, muscles, and skin of gilthead sea bream (Sparus aurata). The contents of P ranged from 1.8 to 13 g per kg, with fish bones showing about 10 g per kg and heads 13 g per kg. The Ca contents in heads and fish bones were high, ranging from 16 to about 24 g per kg [220].

On the other hand, the K contents were generally low in fish bones and other FW, ranging from 0.003 to 1.7 g per kg DM compared to the processed fish, which had higher K contents.

These findings from various studies highlight that fish and fish waste possess significant amounts of minerals, particularly P, Ca, and N, which could be valuable in supplementing the nutritional requirements of crop plants in agricultural systems.

Using fish-based fertilizers can be an environmentally sustainable way to recycle these essential nutrients back into the soil and promote the overall health and fertility of the soil-plant system.

1.5.2 Processing of fish/FW for use as feed or fertilizer

Fish and fish waste (FW) can undergo various processing methods to be converted into stabilized liquid or solid forms of fertilizers. These processed products can be used as fish compost or substrates in anaerobic digestion. Several terms are used to describe FW products depending on the processing method, including fish solubles/fish emulsion, fish soluble nutrients, hydrolyzed waste/fish hydrolysate (also called fish silage), fish meal, and fish powder.

The processing steps vary based on the type of material and can involve grinding, heating, pressing or centrifugation, separation into solid and liquid phases, drying of solid materials, and grinding of dried materials to achieve the desired particle size. Heating is used to coagulate proteins, rupture fat depots, and liberate oil and physiochemically bound water. The liquid phase is separated into oil and stick water (solubles), while the solid phase is used to produce meal. Fish meal is typically produced through heating, pressing, separation, evaporation, drying, and grinding. Different techniques, such as freeze-drying, spray drying, and oven-drying, can be applied for the drying process. Additionally, fish meal can also be produced through fermentation [212].

Fish hydrolysate or fish silage can be further dried or modified to produce fish meal, fish bone meal, or fish powder, which can be used in animal feed or applied as fertilizers. These processed products can also be mixed with other materials to create fish compost, serving as a beneficial organic amendment for soil [211].

Fish meal or fish bone meal can be used in both solid and liquid fertilizer products. For liquid fertilizer applications, the meal should be finely ground to remain in suspension and flow through dispersion nozzles when dispensed through sprayers [212].

In summary, the processing of fish and fish waste offers various ways to convert these materials into valuable fertilizers and composts, contributing to sustainable agriculture practices and nutrient recycling.

1.5.3 Anaerobic digestion of fish waste and possible use of biogas digestate as fertilizer

Anaerobic digestion (AD) is a well-established recycling technology used in biogas plants to manage biodegradable solid waste. During AD, organic matter (substrate) undergoes decomposition in an oxygen-free environment (digesters), resulting in the production of biogas and digestate (digested substrate). Essential plant nutrients such as nitrogen (N) and phosphorus (P) are retained in digestates, making them beneficial as fertilizers and soil amendments [212].

Fish waste (FW) can vary in nature depending on fish species and processing and may contain approximately 20% ether extract or fat, about 60% proteins, and minerals. It also contains sufficient amounts of monounsaturated acids, palmitic acid, and oleic acid (22%). The anaerobic co-digestion of FW with other organic wastes, particularly agricultural wastes, has been recognized as a valuable approach to utilizing FW to generate energy, playing a significant role in the future of biofuels [221].

By utilizing anaerobic digestion and anaerobic co-digestion, the organic matter present in fish waste and other organic materials can be effectively converted into biogas, a renewable energy source, and digestate, which can serve as a nutrient-rich fertilizer for agricultural purposes, contributing to sustainable waste management and resource recovery.

Fish waste (FW) has been successfully applied as a substrate in anaerobic co-digestion with various other materials, showcasing its potential in sustainable waste management and resource utilization. Some of the materials used in co-digestion with FW include cow dung, cattle manure, strawberry exudate, sisal pulp, bamboo residues, and the vegetable fraction of market solid waste [221].

The co-digestion of FW with the liquid fraction of hydrothermal carbonization of bamboo residues has been proposed as an effective strategy for managing complex-to-degrade liquid waste [209]. Additionally, co-digestion of FW with strawberry waste has resulted in a digestate that is rich in nutrients, making it a valuable resource as a nutrient-rich fertilizer. In the United States, a patent has been presented for a process that generates an anaerobic digestate-based fertilizer product. This process involves combining and processing anaerobic digestates with stabilized liquid fish products, likely enhancing the nutrient content and agronomic value of the resulting fertilizer [212].

The utilization of FW in anaerobic co-digestion with various organic materials not only provides an effective waste management approach but also offers a sustainable means of producing biogas as an energy source and generating nutrient-rich digestate that can be beneficial for agricultural purposes.

1.5.4 Scientific studies related to commercially available fish-based fertilizers

The Organic Materials Review Institute (OMRI) has approved a total of 154 commercial fish-fertilizer products. However, scientific research on these products is relatively limited, with only a few studies available. Some of the commercially available fish-based fertilizers that have been investigated in scientific research include fish meal, fish-scale meal, fish pellets, fish-derived protein hydrolysates, hydrolyzed fish extracts, fish emulsion, and fish bone formulations.

Notably, some significant results related to the growth effects on agricultural and horticultural plants, tree species, and berries have been observed in these studies:

- Agro fish pellet positively affected the growth parameters of tomato plants, leading to an increase in stem diameter, shoot dry weight, number of flowers and fruits, fruit yield, and overall fruit quality [222].
- Fish meal and fish-scale meal, when mixed with a peat-compost growing medium, enhanced the shoot weight of greenhouse tomato transplants [223].
- Fish bone meal, either alone or in combination with marine sediments or seaweed + fish oil, was applied over six growing seasons to a 3-year vegetable crop rotation consisting of cabbage, carrot, and green beans. Marketable yields of cabbage and carrots were comparable to yields obtained with standard vegetable mineral fertilizer (NPK: 17–17–17), while yields of beans were better with mineral fertilizer [224].
- HFPC (hydrolyzed fish protein concentrate) hydrolyzed powder supported the colonization of leek roots by arbuscular mycorrhizal fungi. Leek plants treated with fish-based fertilizer had twice the shoot weight compared to plants treated with conventional fertilizer [225].

- Treatment of lettuce plants with fish-derived protein hydrolysates significantly increased the number of leaves, stem diameter, shoot, and root dry weight of lettuce plants [226].
- Hydrolyzed fish fertilizer was used in several studies on calibrachoa and marigold plants. Calibrachoa plants fertilized with fish fertilizer showed relatively low shoot dry weight compared to the mineral fertilizer Plantex (high nitrate 20-2-20). However, the calibrachoa plants were healthy. On the other hand, marigold plants, whether given fish or mineral fertilizer, exhibited high quality [208].
- Application of fish extracts to Chrysanthemum plants promoted leaf growth compared to no fertilizer treatment [227].
- Foliar concentrations of nitrogen (N) in western red cedar trees were highest in trees treated with inorganic fertilizer or fish silage (in combinations with ash). However, the fish silage treatments resulted in smaller growth responses [228].
- Fish emulsion-based liquid fertilizer did not show any positive effect on soil-borne disease incidence and fruit yield, number, and size in organic greenhouse tomato production systems. However, in another study, sandy soil amended with fish emulsion enhanced the growth and development of radish when combined with bacterial or actinomycete mixture treatments [229].

- In studies conducted by Abbasi and coworkers, fish emulsion was applied to tomatoes, radish, cucumber, peppers, and eggplant. Its application enhanced the health and fruit yield of tomatoes and peppers and promoted the growth of radish seedlings [230].

OMRI-approved fertilizer products, such as fish emulsion or feather meal, have been commonly used as nitrogen sources by US organic blueberry farmers. The fish emulsion is applied either as a direct liquid application to the soil or through the drip irrigation system [212].

Therefore, the effectiveness of fish-derived fertilizers can vary depending on the plant species, the specific formulation of the fertilizer, and the application method.

These studies show that a number of commercial fish-based fertilizers have increased the growth of several horticultural and ornamental plants, as well as maize (corn) and young trees. For crops such as cereals, ley, and potatoes, studies with these commercial fertilizers are scarce. This may reflect that these commercial products may be costly to apply in arable crops and leys, or that they may be more suitable for horticultural or ornamental plants. Besides, for many organic vegetable growers, management of N is crucial, especially those producing high N-demanding crops. The application of hydrolysed fish fertilizer as an in-season supplement can be the source of quickly available N.

Thus, hydrolysed fish fertilizer is suggested as a prospective economic option in organic farming [231].

1.5.5 Effect of fish waste-based non-commercial fertilizers on plants

The studies mentioned in the literature search have shown promising results regarding the use fish-based fertilizers, including fish emulsions, liquid fish extracts, and compost derived from fish waste on plant growth and soil fertility in different countries:

- Researchers from the USA applied fish soluble nutrients on various crop plants, including lettuce, radish, sorghum, sweet corn, peas, and soybean, over a period of seven years. Fish soluble nutrients were found to be beneficial, especially when applied with moderate dilution [232].
- In India, liquid fertilizer prepared from fish waste, amended with Bacillus subtilis bacterial culture and sterile water, showed positive effects on the growth of tomato plants compared to diammonium phosphate fertilizer [233].
- The liquid fermented fish product called "Gunapaselam" was tested on soil and different plants. Its application decreased soil pH and improved soil properties, including exchangeable cation levels, organic carbon, organic matter, nitrogen, phosphorus, and potassium content in brinjal (Solanum melongena Linn.) plants. Gunapaselam was

indicated to be a valuable resource for enhancing soil fertility and plant growth [234].

- Liquid fish silage prepared from non-edible parts of tread fin seabream (Nemipterus japonicus) in Malaysia showed comparable plant growth, yield, pigment content, and post-harvest quality in pakchoi (Brassica rapa L. subsp. chinensis) when compared to commercial fertilizer (N-P-K; 15:15:15) [235].
- Fish emulsions were tested on tomatoes, basil, spearmint, and blueberries in the USA, and radishes in Australia and Egypt. The application of fish emulsion resulted in improved plant growth and yields [229].
- Liquid fertilizer containing fishery waste and seabird guano, named Phytamin 801, was applied to fescue turf in California, USA, showing positive effects on the soil properties [236].
- Fish extracts from China were tested on chrysanthemum plants, resulting in enhanced leaf growth [227].
- Compost produced from fish waste mixed with seaweed was found to be a suitable soil amendment for horticultural crops. Its application significantly increased tomato and lettuce yields, as well as potato and maize production, in different studies [237].

- The compost from fish waste mixed with pine bark improved the fresh and dry matter yield of leaves of ice lettuce and affected nutrient concentrations in the soil [237].
- Compost from fish waste composted with crushed grass promoted the growth of lettuce plants through the presence of humic acids [238].

Overall, the studies show that fish-based fertilizers, including liquid fish extracts and compost from fish waste, have the potential to improve plant growth and increase crop yields. The application of such fertilizers can provide essential nutrients like nitrogen and phosphorus to support plant growth and enhance soil fertility, making them valuable resources in organic agriculture.

Utilizing fish waste (FW) as fertilizers in agriculture has significant potential to reduce the dependence on importing phosphate (P) from finite and non-renewable phosphate rock reserves and contribute to sustainable nutrient recycling. As mentioned earlier, FW contains substantial amounts of minerals, including phosphorus, which is a crucial nutrient for crop growth and food production.

Based on the assumption that FW contains 30% dry matter (DM) and 10% of DM is P [212], the annual amount of P available from the 40% of FW that is currently not utilized is estimated to be approximately 3900 tonnes. This is a substantial amount of P that could be harnessed for agricultural use.

Fish bones from FW can be a valuable source of phosphorus. Methods such as solubilization of fish bones or directly using hydrolyzed fish bones for fertilization have been explored [212]. These approaches can convert the phosphorus-rich fish bones into accessible forms of P that can be used as fertilizers to support plant growth. Research and development in this area can further optimize the utilization of FW-based fertilizers and contribute to more environmentally friendly and efficient agricultural practices.

FW-based fertilizers have the potential to improve soil microbial activity, soil structure, and stimulate root growth, leading to enhanced soil health and fertility. Moreover, the utilization of FW for producing fertilizers aligns well with the EU's circular economy policy, as it recycles scarce minerals from the sea back into terrestrial environments.

In organic farming, hydrolyzed fish fertilizers can serve as a valuable source of nitrogen (N) for N-demanding crops, addressing the need for N supplementation in organic agricultural systems.

The versatility of FW processing allows for the production of both liquid and solid fertilizers, and they can be combined with other materials, such as seaweed, to create fish-based composts, digestates, or FW-seaweed fertilizers, enriching the range of options available for organic growers.

With the increasing focus on organic farming in Europe, the abundance of FW provides a promising and sustainable source of raw material that can potentially replace conventional fertilizers, such as dried poultry manure, in organic agriculture.

1.6 Anchovies

The processes involved in raising, catching, and processing fish generate significant amounts of waste, which has become a global concern [209]. For each tonne of fish consumed, approximately an equal amount of fish waste (FW) is discarded through ocean dumping or land disposal. The term "fish waste" encompasses various materials, such as whole fish (dead or damaged fish), fish trimmings, and specific tissues, including heads, intestines, tails and fins, skins, scales, and bones, among others.

Pelagic fish encompass species that inhabit the uppermost layer of the water and can be found at depths of up to 200 meters. They represent a significant portion of the marine fish habitat and play a crucial role in shaping the global marine ecosystem economy [239]. Notably, small pelagic fish contribute substantially to the overall annual world fisheries catch, accounting for approximately 20%-25% of the total [240]. Examples of small pelagic fish include herring, sprats, pilchards, anchovies, sardines, mackerels, carangids, mullets, and ribbon fishes.

Nevertheless, in areas like the continental coast, the anchovy, sardine, and mackerel stand out as the largest species [241].

Anchovies (figure 1.4), specifically, are dominant small pelagic fish found throughout the Indo-Pacific region and play a significant role in both human consumption and serving as feed for larger fish. Classified under the order Clupeiformes and the family Engraulidae, anchovies encompass approximately 16 genera and 172 species. Their distribution spans extensively across the Indian and Pacific Oceans, primarily inhabiting shallow coastal waters. Among the various genera, Anchoa comprises 35 species, Anchoviella consists of 15 species, Stolephorus has 19 species, and Thryssa accounts for 25 species, making up the majority of commonly found anchovy species [242].

Figure 1.4 Anchovies (https://draxe.com/nutrition/anchovies/).

Anchovies are small to moderate-sized fish, exhibiting a length that can range from 8 cm (Stolephorus banganensis) to 32 cm (Setipinna brevifilis) [243]. These fish display a diverse array of colors, often appearing blue/green or brown on the back, with a silver flank and a distinctive bright silver lateral stripe.

According to the Food and Agriculture Organization of the United Nations (2017), anchovies and sardines collectively make up approximately 52% of the global small pelagic fish landings. In Malaysia, the total marine fish landing, including pelagic fish, was reported to be 560,879 tonnes in 2019. Among this amount, anchovy accounted for 28,894 tonnes [244]. The primary landing sites for anchovies in Malaysia are Kelantan, Sabah, and Kedah, with Kedah alone contributing 40% of the total landing. Additionally, anchovy captures are also observed at Pulau Pangkor (Perak) and Pulau Perhentian (Terengganu-Kelantan border). Notably, the peak landing of anchovies typically occurs from the beginning of April to June and from September to October [245].

These fish are captured in substantial quantities in the coastal waters of Malaysia, employing anchovy's purse seines and lift nets as common fishing methods. Within the Southeast Asian region, two dominant species that are frequently caught include the shorthead anchovy (Encrasicholina heteroloba) and the Indian anchovy (Stolephorus indicus), as noted by SEAFDEC (2018). Furthermore, Malaysia's coastal waters frequently yield anchovies from the genera Encrasicholina and Stolephorus, with species such as Encrasicholina heteroloba (bunga air kepala pendek), Encrasicholina punctifer (bunga air), Stolephorus commersonii (bilis tembaga), Stolephorus andhraensis (bilis andra), and Stolephorus indicus commonly found in the area [244].

Fish and other marine animals play a significant role as commodities worldwide. The consumption of fish offers several health benefits, including the prevention of diseases such as blood pressure issues, cancer, and coronary heart disease, primarily due to its rich composition of amino acids and omega-3 highly unsaturated fatty acids (HUFA) like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [242].

Anchovies, in particular, are highly recommended for regular consumption, with the World Health Organization (WHO) and the United Nations Food and Agriculture Organization (FAO) suggesting at least two servings per week. This recommendation is based on their exceptional nutritional composition, which bestows various health benefits. To better understand the nutritional value, chemical changes, and potential reactions during handling, it is crucial to have information about the proximate composition of anchovies. Fish, including anchovies, stand as one of the primary sources of protein in human nutrition, underscoring the importance of studying their proximate composition [242].

The composition of fish, including anchovies, is influenced by various factors such as their diet, feed rate, genetic strain, and age (Sankar et al., 2013). Anchovies offer significant nutritional value beneficial for health. Fish proteins, in general, exhibit higher nutritional value compared to proteins from other animal sources and contain nearly all essential amino acids. Studies have demonstrated the essential role of fish protein in various health aspects, including inflammation, metabolic syndrome, osteoporosis, and cancer. Due to the nutritive value of anchovies, they find application in numerous food products. Understanding the chemical composition of fish, including anchovies, becomes crucial for the development of fish products on a commercial and industrial scale in the food processing technology [242]. Moreover, anchovies have been utilized in various food products, both in traditional culinary practices and in the development of highly processed food items.

1.6.1 Application of anchovies as a food product Literature review

The ten most caught fish species together account for approximately 33% of global fish captures [246]. Among these species, anchovy, particularly the Peruvian anchoveta (Engraulis ringens), stands out as the most heavily caught fish worldwide, representing approximately 11% of the world's total landings. In 2018, around 7.045 million tonnes of Peruvian anchoveta and 0.957 million tonnes of Japanese anchovy (Engraulis japonicus) were harvested [246].

Regarding European anchovy (Engraulis encrasicolus), Turkey is the largest producer, with a catch of 193.492 tonnes in

2015, followed by Spain (49.982 tonnes), Russia (45.683 tonnes), and Italy (as shown in Table 1) [247].

Anchovies are commonly sold either fresh or canned, with canned fillets being a popular form of consumption. The process of preparing canned anchovies involves salting and ripening in either sunflower or olive oil. Salting helps to reduce the water content in the fish and prevent the growth of spoilage bacteria by allowing marine salt to diffuse into the fish. During the ripening process in oil, which takes place under anaerobic conditions to limit the oxidation of polyunsaturated fatty acids abundant in anchovy fat, various biochemical processes driven by enzymes decompose proteins and fats [248]. As a result, soft anchovy fillets are obtained, and they are widely regarded as a gourmet delicacy.

Over the last two decades, there has been a global trend towards increased demand for foods that offer significant health benefits. This demand is influenced by various socioeconomic factors, including a growing population, increased life expectancy, and rising healthcare costs. Anchovies have emerged as an essential component of the human diet due to their provision of essential nutrients, and they are particularly valued for their high-quality protein, surpassing that of meat and eggs. Anchovies hold considerable value as a food source in many Asian countries, and their popularity has contributed to their higher price compared to other food items. Moreover, selected species of anchovies are highly valued as food, while other species find use as baits or as fish meals in various applications. The versatility of anchovies in their utilization further enhances their significance as a valuable resource in the food industry and beyond [242].

1.6.2 Italy's anchovy industry

In Italy, the production of European anchovy experienced a peak in 2006, reaching over 81,000 tonnes, but then sharply declined to a minimum of 29,644 tonnes in 2013 [249]. Since then, the catches have been on the rise again, surpassing the 38,000 tonnes mark in 2017, with a total of 38,151 tonnes harvested [250].

European anchovy, along with European pilchard (Sardina pilchardus), accounts for more than one-third of Italian marine catches in terms of economic value, with anchovies typically fetching higher market prices [251]. The spawning period for anchovies occurs in coastal areas from March to September [252]. Consequently, prices of anchovies, for instance, in Sciacca, the main small pelagic fishing port in Sicily, show an inverse correlation with landings during this period [253]. For context, the anchovy processing industry in Sciacca produces about 5000 tonnes of small pelagic fish annually, generating approximately €30 million in revenues [254]. Alongside the portion of the catch consumed fresh, a significant amount of anchovies is utilized for producing anchovy fillets in specialized plants. In Sciacca alone, over 1000 workers are employed in the anchovy processing industry [254]. This highlights the economic importance of anchovy fishing and processing in Italy, particularly in regions like Sicily.

The impact of marine currents on the socio-economic performance of the fishery is significant, as demonstrated by a recent model that describes the hydrographic variability and biomass fluctuations of European anchovy in the central Mediterranean Sea [255]. This model allows for a monetary estimation of catches based on different transport dynamics.

In summary, a higher anchovy biomass in the central Mediterranean area is observed when there is a favorable combination of advective currents and egg deposition in the previous year. Coastal currents play a crucial role in delivering anchovy eggs and larvae along the shore, from the spawning ground of Sciacca to the recruitment area off Cape Passero, in southern Sicily [255].

Each adult anchovy (1–2 years old) in this region of the Mediterranean Sea weighs approximately 13.2 grams [256], and annual landings show significant variability, ranging from 1000 to over 5000 tonnes. To produce 1 kg of anchovy fillets preserved in oil, it takes 2.25 kg of anchovies [247]. These details underscore the complexity of anchovy fishing and the role of marine currents in influencing the abundance and distribution of anchovy populations, which ultimately affects the catch and economic performance of the fishery.

Indeed, the production of anchovy fillets preserved in oil generates a significant amount of biowaste. For every kilogram of anchovy processed, approximately 0.55 kg of anchovy biowaste is produced, which includes parts of the fish that are not used for the fillets.

In Italy, the production of anchovy fillets preserved in oil amounted to 3951 tonnes in 2016. Among the various companies involved, eight major companies were responsible for around 80% of the national production, equivalent to approximately 3000 tonnes, while smaller companies accounted for the remaining 20% [247].

To handle the disposal of anchovy fillet waste, fish processing companies face economic costs. For instance, in Sicily, as of 2021, the typical tariff paid for disposal is approximately €0.2 per kilogram of biowaste if the composting plant, where the waste undergoes composting along with other biological residues, is located within a 100 km radius [257]. However, if the composting plant is farther away, a substantially higher tariff will be incurred.

Based on the mentioned production rate of anchovy fillets in Italy, which amounted to 3951 tonnes in 2016, it can be estimated that Italian anchovy processing companies purchased approximately 8979 tonnes of fresh anchovies that year. Out of this, approximately 55% (4938 tonnes) was disposed of as biowaste, underscoring the considerable environmental and economic impact of anchovy fillet processing. Finding sustainable solutions for the management of such biowaste is crucial for both the fishing industry and the environment.

1.6.3 Proximate composition of fresh anchovy

The quality assessment of fish involves considering factors such as color, texture, and nutritional composition [258]. Typically, the proximate composition of whole fresh fish comprises approximately 66-81% moisture content, 16-21% protein, 0.2-15% fat, 1.2-1.5% minerals, and 0-0.5% carbohydrates (Mazumder et al., 2008). However, for small pelagic fish like anchovies and sardines, the proximate composition can vary depending on the fishing season (Kudale and Rathod, 2015). Furthermore, the chemical composition of fish is influenced by various factors, including age, sex, environment, fish feed, and species (Boran et al., 2008; Herawati et al., 2018).

Boran et al. (2008) reported that anchovies consist of 65.9–77.9% water, 12.8–19.8% protein, 1.81–15.3% fat, and 1.5–2.3% ash. The high moisture content of anchovies makes them perishable and provides a suitable environment for microbial growth after their death. The moisture content of fresh anchovies has been reported in the range from 75.56 to 81.00 g/100 g [242]. Among the different species, Engraulis anchoita exhibits the highest value of crude protein (22.20 g/100 g), while Stolephorus commersonii displays the lowest value (16.32 g/100 g). The protein content in these species (>15%) classifies them as high-protein fish, consistent with the standard proximate composition of fish protein reported to be between 15-23% (Abraha et al., 2018).

The average protein content of anchovies is comparable to that of Indian mackerel, Yellow stripe scad, Fringescale sardinella, and Spanish mackerel, as reported by Nurnadia et al. (2011). Overall, the proximate composition of fresh anchovies highlights their nutritional significance and underscores their value as a high-protein fish species.

In addition to being rich in protein, anchovies boast a substantial amount of polyunsaturated fatty acids (PUFAs) and phospholipids [259]. The fat content of different species of fresh anchovies has been reported in the range of 1.62 – 3.50 g/100 g [242], with the highest value observed in the species Engraulis anchoita. However, variations in fat content can arise due to factors such as feeding habits and time of catch, the season of capture, sexual maturity, and geographical region [239].

Even within the same species, the fat content can differ based on age variation and sexual maturity. These variations underscore the importance of considering multiple factors when evaluating the nutritional composition of anchovies and highlight the dynamic nature of their fat content in response to different ecological and biological factors. The ash content of fish species typically ranges from 1.0 to 3.92%. Ash represents the inorganic residue left after removing moisture and organic matter through heat treatment and is indicative of the mineral content in food [260].

A higher ash content in fish indicates a higher mineral composition, which is beneficial for human health [261]. However, concentrations of minerals and trace elements in anchovies may vary due to factors such as feeding behavior, environmental ecosystem, and migration patterns [262]. These variations highlight the influence of ecological factors on the mineral content of anchovies.

According to Kari et al. (2022) [242], anchovies have a relatively low carbohydrate content, with reported values below 0.10 g/100 g. Studies on anchovy composition rarely report significant carbohydrate content (Czerner et al., 2011; Abraha et al., 2017; Madathil et al., 2017; Rojas-De-Los-Santos et al., 2018; Reksten et al., 2020). This observation underscores that anchovies are primarily a protein and fat-rich food source, with carbohydrates playing a minor role in their overall nutritional composition.

According to Anthony et al. (2000) [263], the carbohydrate content of fish is reported as significantly low. Hence, the carbohydrate composition in anchovies can be considered insignificant as the estimated value shows a considerable difference from other components. Moreover, the variation of crude protein content, crude fat content and ash content between the species of the anchovies may be related to the feed intake of the fish. Different geographical locations and seasonal changes influence the chemical composition of fish muscle due to the availability of feeds [264]. According to Boran et al. (2008) [265], during the seasons of heavy feeding, the protein content of muscle tissue becomes slightly increased and causes a rapid increase in lipid content. On the other hand, starvation periods in which the period where a decrease in the amount of plankton that is consumed by anchovy can occur. As a result, anchovy may have a variation in its proximate composition.

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Chapter 2

A circular economy approach to fish oil extraction

2.1 Omega-3 extraction from anchovy fillet leftovers with *d*-limonene

The consumption of omega-3 long-chain polyunsaturated fatty acids (PUFA), which are plentiful in oily fish, is crucial for the physical and mental well-being of both adults and children [1]. Health authorities worldwide generally recommend that healthy adults increase their omega-3 fat intake by regularly consuming fish twice a week. If fish consumption is not regular, a fish oil supplement of 2 g several times a week can be taken. Fish oil serves as a primary source of omega-3 PUFA [2].

The main long-chain PUFA belong to either the ω-6 (n-6) or ω-3 (n-3) families, depending on the position of the first double bond from the methyl end group (ω end) of the fatty acid [3]. To restore a better balance between ω-3 and ω-6 fats, the World Health Organization recommends a daily intake of 250 mg of eicosapentaenoic acid (EPA, 20:5 ω-3) plus docosahexaenoic acid (DHA, 22:5 ω-3) for primary prevention of coronary heart disease, and 2 g for secondary prevention [4]. The European Food Safety Authority also recommends a daily intake of 250 mg for EPA plus DHA [5].

Omega-3 nutrients play a fundamental role as hormone precursors, helping to regulate the tendency for excessive arachidonic acid cascade reactions when n-6 mediators dominate [6]. The composition of ω-6:ω-3 in tissues affects their ability to defend against oxidative stress. A high percentage of omega-6 can lead to persistent inflammation, further intensified by the continuous generation of free radicals. On the other hand, omega-3 fats reduce the levels of prostaglandins 2-series PG, which are potent mediators of inflammation and cell proliferation, while promoting the synthesis of 3-series PG, which are less inflammatory in nature.

In a recent study focused on improving methods for extracting omega-3 fats from fish oil, it was concluded that there is an urgent need for practical and sustainable advancements in the extraction and sourcing of marine omega-3 nutrients [7]. This is crucial because new health benefits associated with omega-3 intake, extending to the prevention of various diseases, are continuously being reported [8].

Furthermore, the unsustainable fishing practices specifically targeting anchovies, which are a preferred source of omega-3 fats, have resulted in concerns regarding the availability and size of anchovy populations. Therefore, the Peruvian government took measures such as suspending one of the two annual fishing seasons in 2014 and implementing quotas for the first anchovy fishing season in 2017, even while the previous season was still ongoing [9].

The global volume of EPA/DHA ingredients has experienced a significant increase, reaching 91,321 tonnes in 2016 from 87,925 tonnes in 2015, driven by the growing demand in Asia [10]. Peruvian anchovies are the primary source of oil used for producing dietary supplements, despite the fact that only approximately 5% of the global fish oil production is dedicated to extracting omega-3 nutrients for use in food and dietary supplement products. The majority of fish oil is utilized as fishmeal for fish farming purposes [7].

In the conventional production process of fish oil omega-3 concentrate, anchovies are cooked and pressed on board the shipping vessel. The extracted water-oil mixture is then transported to industrial sites for further processing. At these sites, the mixture undergoes a series of refining steps to remove water. This includes the use of a three-phase centrifuge. Subsequently, the oil goes through additional refining processes such as alkali neutralization, bleaching, deodorization, and degumming.

The resulting refined fish oil typically contains approximately 30% omega-3 fatty acids, with 18% being EPA and 12% being DHA. Omega-3 supplements are produced using either 55% omega-3 ethyl ester oil, where the triglycerides from natural fish oil are transesterified with ethanol and then subjected to molecular distillation, or highly concentrated fish oil extracts that provide 70% active ingredients [7].

The latest methods for obtaining omega-3 dietary supplements involve the use of supercritical fluid extraction combined with supercritical fluid chromatography or enzymeassisted concentration. These techniques allow for the conversion of omega-3 ethyl esters back into the triglyceride form. It is worth noting that the most effective omega-3 dietary supplements currently available contain EPA and DHA in triglyceride form. When consumed as triglycerides, these supplements lead to a 70% higher increase in the omega-3 index compared to when consumed as ethyl esters [11].

Expanding the production of omega-3 from blue fish to fishery by-products that are typically discarded as waste presents an opportunity to recover and utilize essential nutrients from the sea, bringing significant economic, environmental, and health benefits. During fish filleting, as much as 60% of the fresh fish is often cut off and treated as waste. Additionally, a significant quantity of by-products from the blue fish and seafood industry, including heads, skins, trimmings, and bones, are typically discarded back into the sea. However, the potential of these marine processing by-products for omega-3 production has long been recognized [12].

Widely practiced on an industrial scale, primarily for the production of omega-3 lipid food supplements, fish oil extraction involves various marine species, encompassing not only oily bluefish but also krill and crustaceans [13]. This process is succeeded by thorough refinement of the extracted oil and typically results in fish oil in the synthetic ethyl ester form, as opposed to its natural triglyceride form [13].

For decades, diets in most industrialized nations have been deficient in anti-inflammatory docosahexaenoic acid (DHA, C22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3) omega-3 (or n-3) long-chain polyunsaturated fatty acids (PUFA), which are abundant in fish oil, creating a global hidden hunger issue [14]. This, in turn, significantly contributes to overfishing, with even krill now facing overfishing pressures driven by the demand for omega-3 food supplements [15].

Extensive research efforts have been devoted to replacing the current unsustainable methods of fish oil production with circular economy processes that utilize seafood processing biowaste [13][16] and greener, more efficient extraction methods [17]. Recent estimates suggest that optimizing omega-3 supply through the replacement of marine species with higher omega-3 content, promoting direct consumption of wild fish, and improving by-product utilization to reduce waste could potentially increase the availability of fish oil by 50%, reaching 630 thousand tons per year [18].

Within these new, environmentally friendly circular economy extraction methods, the "LimoFish" process employs orange oil-derived *d*-limonene as the extraction solvent of a whole fish oil named “AnchoisOil” from Mediterranean anchovy fillet leftovers, shifting omega-3 lipid production from fish to fish processing waste. Initially demonstrated with European anchovy (Engraulis encrasicolus) fillet remnants [19] and subsequently extended to shrimp biowaste [20], this process proves to be economically and technically feasible [21] while also being environmentally sustainable [22]. Furthermore, this method closes the material cycle for anchovy fishing, as the solid residue of anchovy fillet remnants, extracted with antibacterial and health-beneficial limonene [23], serves as a highly effective organic fertilizer known as "AnchoisFert" [24][25].

These findings hold immense potential to significantly enhance the sustainability of anchovy fishing, omega-3 lipid production, and fertilizer production, given that anchovy constitutes 11% of the world's total fish landings, with Peruvian anchovy (Engraulis ringens) being the most captured fish species worldwide [26].

2.2 Extraction Process and fish oil analysis

Anchovies are among the most heavily fished species worldwide, and their population is at risk due to overfishing [27]. The European anchovy (Engraulis encrasicolus) is particularly abundant in the Sicilian Channel, and its capture for the production of anchovies filleted, salt-cured, and stored in olive oil is a vital economic resource for urban centers in southern Italy. This includes the city of Bagnara Calabra, which is where the anchovy fillet leftovers (Figure 1.1) used in this study were generously provided by a company specializing in the sale of anchovy fillets. Fish oil was extracted from industrial waste generated during anchovy filleting using *d*-limonene, a green bio-solvent, following the solid-liquid extraction and solvent recovery processes described in a published study [28]. The extraction process involved the use of an electric blender to mix and homogenize the frozen anchovy leftovers along with a measured amount of *d*-limonene (Figure 2.1).

Figure 2.1 Frozen anchovy leftovers in a glass beaker.

A sample of the frozen waste and the first aliquot of *d*-limonene, cooled at 4°C, were combined in the blender jar. The mixture was then ground to obtain a semi-solid grey puree, which was subjected to extraction using *d*-limonene.

A portion of this mixture was transferred to a glass beaker and mixed with another aliquot of chilled *d*-limonene. A simple solid-liquid extraction was performed by stirring the sealed beaker, covered with aluminum foil and parafilm, using a magnetic stirrer at a speed of 700 rpm for 21 hours at room temperature (Figure 2.2).

The resulting yellow supernatant (Figure 2.3) was collected and transferred to the evaporation flask of a rotary evaporator equipped with a vacuum pump. The solvent was removed under reduced pressure (40 mbar) at a temperature of 90°C.

Figure 2.2 Fish oil solid-liquid extraction by stirring at room temperature.

Figure 2.3 Fish oil extraction yellow supernatant.

The pure limonene was successfully recovered through evaporation under reduced pressure (Figure 2.4) and was ready to be used for subsequent extraction runs. Following the evaporation process, we obtained approximately 3.0 g of fish oil (Figure 2.5), which had an orange color and emitted a pleasant and delicate odor.

Figure 2.4 Solvent evaporation process with rotary evaporator.

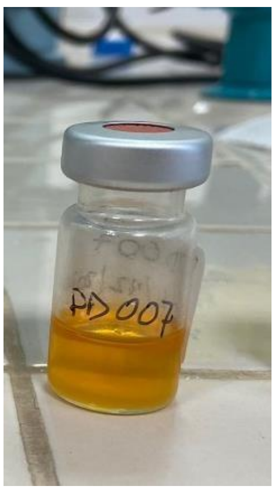

Figure 2.5 The anchovy leftover oil obtained after evaporating *d*-limonene under reduced pressure.

The fatty acid composition of anchovy discard fish oil (Figure 2.5) was assessed following the standard method involving trans-esterification of the oil triglycerides and GC-MS/GC-FID analysis of the respective fatty acid methyl esters.

For the fatty acid analysis, a 100 mg sample of the latter oil was added with 2 mL of Heptane (CHROMASOLV™, for HPLC, ≥99%). The fatty acids in triglyceride form were trans-esterified to obtain the fatty acid methyl esters (FAME) required for the GC-MS analysis by treating the fat residue with concentrated KOH dissolved in MeOH. Most residual limonene dissolved in the methanol liquid phase.

Quantification of fatty acid methyl esters (FAMEs) in fish oil was performed with GC-FID while identification was performed with pure standards (Mix FAME) run at GC-FID and GC-MS through the NIST Mass Spectral Library.

- The GC-FID analysis was carried out using a Shimadzu GC 2010 plus tracera equipped with a flame ionization detector.

A 0.2 µL sample of the FAME solution was injected in the GC (split ratio 148) using a Mega-10 column (Mega, 100 m 0.25 mm id 0.20 um film thickness) with an autosampler AOC-20i Shimadzu, using He (6.0) as gas carrier (flow-rate 0.52 mL/min). The temperature ramp used was as follows: the column was held for 8 min at T = 165°C, after which temperature was raised at 2°C/min rate up to 210 °C, with a final 45 min isotherm. The overall time for the analysis was 75.50 min. Injector and detector temperatures were both set at 250 °C.

The FAMEs were identified by comparing their retention times with those of standard samples (NIST 26 Component FAME Mix, SRM 2377).

- The GC-MS analysis was carried out using a Shimadzu single quadrupole GC/MS QP2010 Ultra spectrometer.

A 1 µL sample of the FAME solution was injected in the GC (split ratio 38) using a SP-2380 column (Supelco, 100 m 0.25 mm id 0.20 um thickness) with an autosampler AOC-20i, using He (6.0) as gas carrier (flow-rate 0.59 mL/min). The temperature ramp used was as follows: the column was held for 8 min at T = 165°C, after which temperature was raised at 2°C/min rate up to 210 °C, with a final 45 min isotherm. The overall time for the analysis was 75.50 min. The injector was 250 °C. Following automatic tuning, the electron multiplier voltage was set at 70 eV. Full scan data were acquired over the m/z range 20–500 at 0.30 s per scan, keeping the ion source at 270 °C.

The retention times and molecular fragment mass data obtained were processed using the instrument's software. All FAMEs compounds were identified by critical comparison with mass spectral data from NIST 11 Mass Spectral Library. The FAMEs were also identified by comparing their retention times with those of standard samples (NIST 26 Component FAME Mix, SRM 2377).

The marine oil extracted from anchovy waste using *d*-limonene was found to be abundant in omega-3 fatty acids, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as shown in Table 2.1.

The lipid profile of different oils was characterized using GC_MS/GC-FID. By comparing the lipid profile of this oil, obtained from waste processing of anchovies with *d*-limonene, with that of oils extracted by cold n-hexane and Soxhlet method (with petroleum ether) from the same matrix, it was found that the process utilizing *d*-limonene is an equally valid technique for producing a fish oil rich in essential fatty acids.

In addition to being a rich source of omega-3 polyunsaturated fatty acids (PUFA), anchovies contain a diverse range of nutrients. These include saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), vitamins such as vitamin E (in the form of α-tocopherol), retinol (vitamin A), vitamin D, and D3 cholecalciferol, as well as protein, amino acids, and minerals [29].

Based on the GC analysis of the leftovers of European anchovies caught in the Sicilian Channel in late April, the major saturated fatty acid found was palmitic acid, followed by myristic acid and pentadecanoic acid. The most abundant monounsaturated fatty acid present was oleic acid. Among the omega-3 polyunsaturated fatty acids, DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) were the most abundant, followed by stearidonic acid and alpha-linolenic acid [29].

The fat content found in the oil extracted from anchovy fillet by-products is comparable to the average fat content of 2.27% found in the whole-body of anchovies caught in the Adriatic Sea [30]. Notably, researchers in Croatia observed significant seasonal variations in the fat content of anchovies, which showed an inverse correlation with water content. The fat content ranged from 0.86% in February to 4.47% in October [30].

Table 2.1 EPA and DHA in fish oil extracted from anchovy waste with *d*-limonene, cold-extraction with n-hexane and Soxhlet extraction.

Polyunsaturated Fatty Acid (in lipid numbers)	*Abundance AnchoiOil*	*Abundance AnchoiOil extraction with n-Hexane*	*Abundance AnchoiOil Soxhlet extraction*
EPA (20 : 5, *n*-3)	**11%**	**10%**	**3%**
DHA (22 : 6, *n*-3)	**15%**	**22%**	**5%**

The use of *d*-limonene as a green solvent for lipid extraction, as an alternative to n-hexane, has been extensively studied by the Chemat's team [31]. It has been established that *d*-limonene offers several technical, environmental, and health advantages over the use of n-hexane [32].

In the early 1950s, Henry Schulz introduced *d*-limonene in Florida and created the first large-volume markets for this isomer. It was marketed as a versatile solvent alternative to toxic solvents commonly derived from fossil fuel sources [33]. Initially, cleaning products for both industrial and household purposes represented the largest market for *d*-limonene.

Environmentally friendly *d*-limonene has proven to be an effective replacement for toxic solvents such as toluene, n-hexane, and chlorinated organic solvents in various industrial applications (see Table 2.2) [34].

Table 2.2 Relevant properties of *d*-limonene and commonly employed organic solvents.

Properties	*n-Hexane*	*Toluene*	*Dichloro-methane*	*d-Limonene*
Boiling point (°C)	68.7	110.6	40	175.5
Density	0.66	0.87	1.32	0.84
Toxicity	High	High	High	Absent
Environmental impact	High	High	High	Absent

An interesting example of using *d*-limonene as a bio-based solvent is its application in the extraction of rice bran oil. In this process, *d*-limonene is used at its boiling point (176 °C) to extract the oil from rice bran. Despite the absence of antioxidants during the recovery step of *d*-limonene through vacuum evaporation at 90°C, the amount of oxidation products in the recovered *d*-limonene is less than 1% of the original biosolvent. This indicates a minimal degree of oxidation, allowing for easy reuse of *d*-limonene [35].

In the case of the extraction process with anchovy leftovers, it is conducted overnight at room temperature. The anchovy leftovers are added to cold *d*-limonene, which is kept at 4°C in a refrigerator. This further minimizes the oxidation of the *d*-limonene, preserving its quality. The recovered biosolvent can be reused extensively, even for extracting anchovy leftovers that have been stored at -20°C for several months. This demonstrates the robustness and reusability of *d*-limonene as a solvent in this extraction process.

The orange color observed in the oily extract of anchovy leftovers is likely due to the presence of retinol and α-tocopherol, both of which are abundant in anchovies [36]. α-tocopherol is the form of vitamin E that is labeled with the E307 food ingredient E number in Europe. It is preferentially absorbed and accumulated in the human body, where it is responsible for several health beneficial effects [37].

An interesting finding is that upon trans-esterification of the solid fat residue with potassium methylate and subsequent extraction of fatty acid methyl esters (FAME) using n-hexane for GC-MS analysis, retinol and α-tocopherol accumulate in the glycerol/methanol layer at the bottom (Figure 4). This enables easy recovery and analysis of these compounds from that layer.

Ciriminna et al. [38] conducted a study where they identified and assessed the amount of fat-soluble vitamins in anchovy oil extracted using *d*-limonene from anchovy fillet leftovers. They utilized the UHPLC-HESI-MS advanced mass spectrometry analytical technique. The study revealed that only vitamin D3 was present in the anchovy by-product oil. The combined quantity of the three isomers of vitamin D3 was found to be 0.0815 μg per gram of oil, which corresponds to a content of 81.5 μg/kg. This is consistent with the typical amounts of vitamin D3 found in fish oils, ranging from 18 to 350 μg/kg.

Fish and fish products are widely recognized as important food sources of vitamin D3. Considering the global deficiency of both vitamin D and omega-3 nutrients in populations worldwide, there is a growing concern for public health. Therefore, it is likely that, with further optimization of the method, new dietary supplements could be developed using fish oil extracted from by-products of blue fish. This straightforward process, based on the utilization of edible and health-beneficial limonene derived from waste orange peel, shows promise in addressing these nutritional concerns [39][40].

In conclusion, we have discovered that high-quality fish oil, rich in omega-3 nutrients, can be efficiently obtained from discarded anchovy fillets using *d*-limonene as a green extraction solvent. The process involves simple solid-liquid extraction at room temperature, followed by the removal of limonene through evaporation under reduced pressure.

Limonene, the main component of orange essential oil widely used in the food industry, is an ideal solvent for producing omega-3 extracts from fish and seafood processing waste. While our method was demonstrated using anchovies, which are among the world's largest fish catches, it can be extended to other fish processing waste, which is available in significant quantities worldwide [41]. Examples of anchovy species include European anchovy (Engraulis encrasicolus), Peruvian anchovy (Engraulis ringens), Japanese anchovy (Engraulis japonicus), and southern African anchovy (Engraulis capensis).

In addition to its application in fish processing, *d*-limonene has shown potential in other areas. In 2015, it was found to have significant activity against Anisakis larvae in vitro, suggesting its potential use in the industrial marinating process [42]. Furthermore, researchers in New Zealand investigated its use in masking the unpleasant fishy odor by co-encapsulating it with fish oil in milk protein microcapsules. The resulting microcapsules containing limonene exhibited improved flavor and odor profiles compared to fish oil microcapsules alone [43].

D-Limonene possesses antimicrobial, antifungal, antioxidant, anti-inflammatory, and anticarcinogenic properties, making it an increasingly important resource in the bioeconomy [44]. As limonene is derived from waste orange peel, its renewable nature aligns with the principles of circular economy and bio-based production processes [45]. By utilizing limonene and fish processing waste, our method establishes a circular economy process, closing the materials cycle and offering significant economic, social, and environmental benefits.

2.3 High stability of AnchoisOil extracted with *d*-limonene from anchovy fillet leftovers

Leftover salted anchovy fillets, which rapidly spoil due to factors such as the presence of NaCl and oxygen, as well as the activation of blood leucocyte peroxidase after evisceration [46], are typically treated as biowaste by anchovy fillet manufacturers. Disposing of biowaste involves significant financial costs, despite the fact that anchovy fillets are sold at a substantial price that could easily cover these expenses [47]. In essence, a potential source of high-quality fish oil and fertilizer goes to waste, usually ending up in landfills or, at best, being used for biogas production [48].

Another significant challenge in the current process of refining fish oil for omega-3 food supplement production is the removal of lipid-soluble marine polyphenols and valuable vitamins that protect omega-3 lipids from rapid oxidation. This necessitates the addition of costly natural antioxidants like tocopherol or olive biophenols by manufacturers [49].

In addition to n-3 PUFAs and oleic acid in its natural triglyceride form, “AnchoisOil” also contains substantial amounts of the three vitamin D isomers (cholecalciferol, calcidiol, and calcitriol) at 81.5 μg/kg [24]. However, anchovy oil also contains other fat-soluble vitamins that contribute to its distinct orange color.

Recent research conducted by scholars in South Korea, using Japanese anchovies (Engraulis japonica), revealed that these vitamins include vitamin A, vitamin E, and vitamin Q (coenzyme CoQ10) [50].

To elaborate further, when comparing oils extracted using supercritical CO_2, *n*-hexane, and a commercial anchovy oil, the research team found that the anchovy oil obtained with supercritical CO_2 had similar acid value, omega-3 lipid content, retinol content, and peroxide value to that of oil extracted with hexane. However, the oil extracted with supercritical CO_2 contained significantly higher levels of vitamins D (over 10 times higher), E (over 12 times higher), and coenzyme Q10 (1.62 times higher) compared to the oil extracted with *n*-hexane at 40°C. Additionally, the oil obtained using non-toxic supercritical CO_2 exhibited a 1.23-fold higher brightness value compared to conventionally sourced dark-colored oil using organic solvents.

Consequently, the research team concluded that fish oil extracted with supercritical CO_2 is "expected to be used as a functional material, which could lead to economic benefits through the high valorization of anchovies" [50].

By utilizing the ultra-high performance liquid chromatography (UHPLC) technique in conjunction with heated electrospray ionization mass spectrometry (HESI-MS), our analytical approach was unable to detect either vitamin A (all-trans-retinol) or vitamin E (alpha-tocopherol) in an AnchoisOil sample extracted from frozen fillet leftovers that had been stored for one year [24].

The objective of this paragraph is to investigate the stability of an AnchoisOil sample obtained from fresh frozen fillet leftovers, which had been subsequently stored for more than four years under nitrogen at -20°C. To achieve this goal, we employed a novel HPLC method capable of simultaneously identifying and quantifying vitamins within AnchoisOil.

The oil extraction process followed the previously described method [38]. The leftover anchovy fillets were sourced from a manufacturer specializing in anchovy fillets in Sciacca, Sicily, which processes anchovies captured in the Strait of Sicily. These leftovers were obtained from the manufacturer and initially stored for a few days at -20°C. Subsequently, they were ground using a mechanical blender and combined with limonene at 4°C. The mixture was stirred overnight. It's noteworthy that the residual amount of limonene in the AnchoisOil, which was isolated after removing the solvent through rotary evaporation at 4 mbar using a water bath at 90°C (until no further separation was observed), was approximately 8% by weight. The analysis of fat-soluble vitamins, namely vitamin A, E, D, and Q, was conducted using High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD). The HPLC system employed was an Agilent 1260 Infinity system (Agilent Technologies, Palo Alto, USA), which included a Binary

Pump G1312C, a Diode Array Detector G7115A, and a column oven G1316A.

Figure 2.6 AnchoisOil samples after more than four years storage under N_2 at -20℃.

Successful chromatographic separation was achieved using a Chromolith Performance RP-18e column measuring 100 mm x 4.6 mm (Merck KGaA, Darmstadt, Germany), which was maintained at a constant temperature of 30°C throughout the analysis.

Before analysis, each sample underwent preparation by adding 200 µL of 2-propanol, followed by filtration through a 0.45 µm PTFE filter to ensure sample purity. The injection volume for each sample was set at 20 µL. The mobile phase consisted of MilliQ water (solvent A) and a mixture of acetonitrile and 2-propanol in a 50:50 ratio (solvent B), both of which were supplemented with 0.1% (v/v) trifluoroacetic acid (TFA).

Solvents were procured from Sigma Aldrich (Milan, Italy), and TFA was obtained from VWR (VWR International, Milan, Italy). The flow rate was fixed at 1.5 mL/min, and the Diode Array Detector (DAD) was configured to cover a wavelength range spanning from 200 to 800 nm. The chromatographic elution was executed following this program: initially, an isocratic condition of A:B = 80:20 was maintained for the first 2 minutes, followed by a gradient shift from 2 to 42 minutes, transitioning from A:B = 80:20 to A:B = 10:90. Subsequently, isocratic elution was sustained at A:B = 10:90 from 42 to 45 minutes, with a final gradient from 45 to 50 minutes, returning to the initial running condition.

The analytical standards used were coenzyme Q10 ((2,3-dimethoxy-5-methyl-6-decaprenylbenzoquinone, purity, 98%), vitamin A (retinol, purity, 97.5%), vitamin D3 (cholecalciferol, purity, 98%), and vitamin E (alpha-tocopherol, purity, 96%), all sourced from Sigma Aldrich (Milan, Italy). The retention times for the analytes were as follows: coenzyme Q10 at 16.74 minutes, vitamin A (retinol) at 28.30 minutes, vitamin D3 (cholecalciferol) at 38.53 minutes, and vitamin E (tocopherol) at 39.16 minutes. We established precise calibration curves for both coenzyme Q10 and vitamin A to facilitate the accurate quantification of these compounds. For coenzyme Q10, the λmax (wavelength of maximum absorbance) was 275 nm (linearity range: 0.1375–13.75 µg/mL, regression equation: Area = 23.992 + 59.34 × [µg mL^{-1}], R = 0.999). For vitamin A, the λmax was 325 nm (linearity range: 0.1-10 µg/mL, regression equation: Area = 20.132 + 90.762 × [mg mL^{-1}], R = 0.998). Quantification data are expressed as µg of each analyte in 100g of the respective oil and reported as means of nine measurements (n = 9) ± SE.

The 3D plot depicted in Figure 2.7 provides a clear visual representation of the complex array of compounds within the AnchoisOil extract. This graphical representation serves to underscore the robust extractive capabilities of *d*-limonene, an environmentally friendly solvent widely recognized for its efficacy in extracting natural products [51].

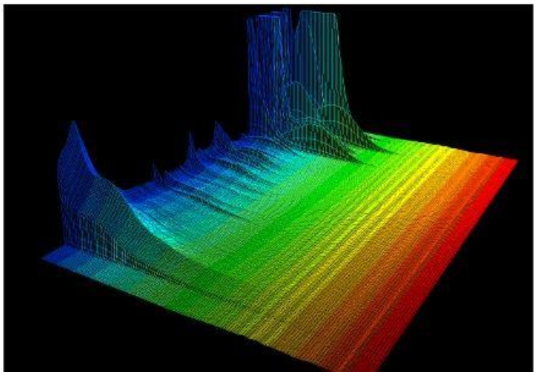

Figure 2.7 3D HPLC plot after injection of AnchoisOil sample after more than four years storage under N_2 at -20℃. Range from 200 nm (violet) to 500 nm (red).

The results of the HPLC analysis for vitamins A, D, E, and Q are presented in Table 2.3.

Vitamin A (μg/100 g)	Vitamin D	Vitamin E	CoQ10 (μg/100 g)
113.4 ± 4.1	n.d.	n.d.	1015 ± 19.1

Table 2.3 Concentration of vitamins A, D, and CoQ10 in AnchoisOil sample more than four years old stored at -20°C.

Each value in Table 2.3 represents the average of nine HPLC measurements. A notable observation from these analyses, when compared to the mass spectrometry analysis conducted on AnchoisOil extracted from frozen fillet leftovers that had been stored for one year [38], is that vitamin A experiences degradation during the storage of anchovy fillet leftovers for one year. Specifically, this vitamin, which is abundant in anchovy oil extracted with supercritical CO_2 (22.51 μg/100 g) or with *n*-hexane (21.92 μg/100 g) but absent from commercial anchovy oil, was not detected after the fillet leftovers had been stored for one year at -20°C.

Conversely, the amount of vitamin A present in AnchoisOil extracted from fresh anchovy fillet leftovers is nearly five times higher (113.4 μg/100 g) than in anchovy oil extracted with *n*-hexane from Engraulis japonica, even after more than four years of storage at low temperatures [50]. This indicates a significant difference in the stability and content of vitamin A between the two extraction methods and storage conditions.

The absence of vitamin E in the aged AnchoisOil suggests two possible scenarios. First, it's possible that vitamin E, which dissolves in limonene under reflux (at approximately 176°C), may not be effectively extracted by limonene at the lower temperatures employed in the LimoFish process. Alternatively, it could indicate that vitamin E undergoes degradation after prolonged storage (>4 years) of the oil, similar to what seems to have happened to vitamin D3, which could not be detected via the HPLC method developed for this study.

In contrast, anchovy oil extracted using hexane under reflux contains a substantial amount of δ-tocopherol, with a concentration of less than 9,000 μg/100 g [50].

Regarding vitamin Q (ubiquinone or coenzyme Q10), its concentration exceeding 1015 μg/100 g is comparable to that found in anchovy oil obtained using hexane under reflux, which is at 1260 μg/100 g [50]. This substantial concentration can be attributed to both the excellent solubility of CoQ10 in limonene [53] and the fact that extraction from anchovy leftovers takes place from regions of the anchovy body, such as the head and viscera, which are particularly rich in this electron carrier that plays a crucial role in mitochondrial energy production and synthesis.

These findings provide further support for the use of this highly bioactive natural fish oil in the prevention and potentially the treatment of diseases. The bright color and transparency of AnchoiOil, which is similar to the color of fresh anchovy oil extracted with supercritical CO_2, even after more than 4 years of storage (Figure 2.6), clearly indicate the absence of degradation in the oil's lipids and its orange-colored vitamins Q and A.

Ubiquinone, also known as coenzyme Q10, is widely utilized as an adjunctive therapy in cardiovascular and neurodegenerative diseases, as well as mitochondrial myopathies [54]. It is a common component of numerous food supplements. It's worth noting that AnchoisOil, when heterogenized over mesoporous silica particles, has demonstrated significant in vitro anticancer activity [55][56].

The absence of vitamin A in AnchoisOil extracted from fillet leftovers aged for one year at -20°C [24] suggests that, for practical applications, the optimal extraction of high-quality AnchoisOil should occur from freshly obtained fillet leftovers, preferably directly at the anchovy fillet manufacturing company [57]. This approach would ensure the preservation of key vitamins and the overall quality of the oil for various potential uses, including dietary supplements and therapeutic applications.

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Chapter 3

Use of the sludge generated during the extraction of fish oil as a nitrogen supplement in anaerobic digestion

3.1 Fish oil extraction residue as substrate in anaerobic digestion

The color of a sustainable future is blue and offers an "ocean" of opportunities. The preservation of oceans, seas, rivers and coasts together with the valorization of aquaculture and marine resources is an important part of the circular economy, creating new chances for a sustainable and inclusive growth [1][2][3]. This approach takes form in the new and ambitious concept of the "blue economy", a paradigm founded on the biomimicry and on the sustainable exploitation of marine and natural resources, which calls for a collective responsibility in preserving the marine environment as one of the key factors of global prosperity [4].

In practice, action for improvement requires the design of innovative production and consumption methods with a far lower environmental impact. Similar efforts are required by the 14th sustainable development goal ("Conserve and sustainably use the oceans, seas and marine resources for sustainable development") of the United Nations 2030 Agenda for Sustainable Development [5][6][7]. In this context, the reuse and valorization of the fish waste is a key process which aims to reduce methane emissions related to unsustainable management (e.g., landfilling) [8]. Furthermore, new economic opportunities for fisheries and the marine/maritime sectors need to be found. Around 35% of the global catch is either lost or wasted every year, whereas about 70% of processed fish turns into by-products (heads, viscera, skin, bones and scales) and is usually disposed of as waste [5]. This biowaste should rather be converted into high value bioproducts and biofuels. While the biorefinery of lignocellulosic biomasses relies, so far, on well-established technologies [9], the complete upgrading of fish waste into biofuels and value-added chemicals can be considered at an embryonic state. Nevertheless, the number of contributions in this field of research are rapidly growing [10][11][12].

Certain fishery by-products are an important source of nutraceuticals and bioactive ingredients [12][13]. Fish by-products are rich in proteins and omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs) [14]. A sufficient daily intake of omega-3 marine essential lipids offers several health benefits to both adults and children and is required for the prevention of many pathologies [15][16]. As a consequence, a significant amount of the global fish catch (22 million tonnes, about 12% of the total) is used for non-food purposes, with about 1 million tonnes of fish oil produced in 2020. Fish oil, at the industrial scale, is produced with established extractive technologies including wet pressing and extraction, with either organic solvent or supercritical CO_2, followed by numerous purification steps [17]. Omega-3 concentrates, for instance, are supplied in the form of synthetic ethyl esters to which natural or synthetic oxidants are usually added to prevent quick oxidation and autooxidation of the double bonds in the LC-PUFA molecular chain.

Recently, a new green process for the recovery of a natural oil rich in omega-3, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and vitamin D3 was developed by the Pagliaro research group, starting from the anchovy processing waste [10]. The method employs citrus-derived *d*-limonene as a non-toxic and edible extraction solvent in a closed-loop process in which the biobased solvent is fully recovered and recycled after the extraction [18]. The residual product, derived from this new extraction process (anchovy sludge), needs to be valorized in order to close the material cycle.

Anaerobic digestion is widely recognized as one of the most effective biorefinery technologies for the upgrading of different types of organic waste and biomasses into biogas through a series of biochemical reactions (hydrolysis, acidogenesis and methanogenesis) occurring simultaneously in an oxygen-free environment [19][20][21]. Together with biogas (methane: 55–70%; carbon dioxide: 30–45%; others: CO, H_2S, NH_3, H_2O, etc. [22]), a solid–liquid residue, generally known as "digestate", is produced [23]. The digestate contains macro- and micronutrients, making this by-product suitable as a sustainable replacement for agricultural fertilizers [24][25]. The sustainable production of biogas through anaerobic digestion (AD) of fish processing waste is an emerging field of research. Eiroa et al. tested the digestion of different fish wastes achieving an average methane yield of 260 $mLCH_4 \cdot g_{VS}^{-1}$ (where VS stands for volatile solids) for tuna, sardine and needle fish waste, and reaching 350 $mLCH_4 \cdot g_{VS}^{-1}$ for mackerel [26]. Nges et al. [27] and Bucker et al. [28] measured a methane yield of 828 $mLCH_4 \cdot g_{VS}^{-1}$ and 540 $mLCH_4 \cdot g_{VS}^{-1}$ for salmon heads and carp viscera, respectively. When anchovy waste was used as starting substrate for anaerobic digestion, a very low methane yield was registered as a consequence of the excessive accumulation of ammonia [29].

In general, the production of methane, through AD processes, ranges from 200 to 900 $mLCH_4 \cdot g_{VS}^{-1}$, depending on the nature of the fish waste [30]. To the best of the authors' knowledge, studies on the use of fish oil extraction residues in anaerobic digestion are virtually non-existent in the literature. Even if this biowaste is rich in lipids and proteins, the concomitant presence of LC-PUFAs, light metal ions (e.g., Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}) and nitrogen-containing species (i.e., ammonia arisen from protein hydrolysis) can inhibit methanogenesis [31]. Furthermore, the residual limonene from the extraction process (and its dehydrogenation product p-cymene) is a well-known inhibitor of anaerobic digestion, and therefore, it is necessary to verify the suitability of the extraction of the solid residue and its subsequent AD process [32][33][34]. In this study, we present, for the first time, biogas production through the anaerobic digestion of the anchovy sludge residual from the extraction of fish oil, produced from the anchovy processing waste using *d*-limonene as a solvent (Figure 3.1).

The produced biomethane can be converted to energy by very efficient combined heat and power schemes, allowing not only to cover the energy demand of the process itself but also to sell surplus energy. In the proposed extraction process, anchovy sludge (AS), as a by-product, is obtained in considerable amount (97% w/w of the total residue quantity) [35]. AS is expected to be rich in *d*-limonene and poor in nutrient elements after oil extraction and, thus, unsuitable for animal feeding or other similar forms of valorization. The advancement proposed and presented in this paper is the evaluation of the potential of by-products derived from fish oil extraction (AS), to produce biomethane by anaerobic digestion, both by batch, with and without previous pre-treatment of the substrate, and by semi-continuous experiments, without any previous pre-treatment.

In particular, semi-continuous experiments afford for a preliminary optimization of the process, solving, at least partially, two of the problems linked to the use of AS a substrate for anaerobic digestion: the high presence of *d*-limonene (whose inhibition capacity during anaerobic digestion is well known [36][37]) and the unbalanced C/N ratio. As discussed below, granular activated carbon (GAC) was used to reclaim and improve the anaerobic digestion processes in a reactor showing clear signs of inhibition.

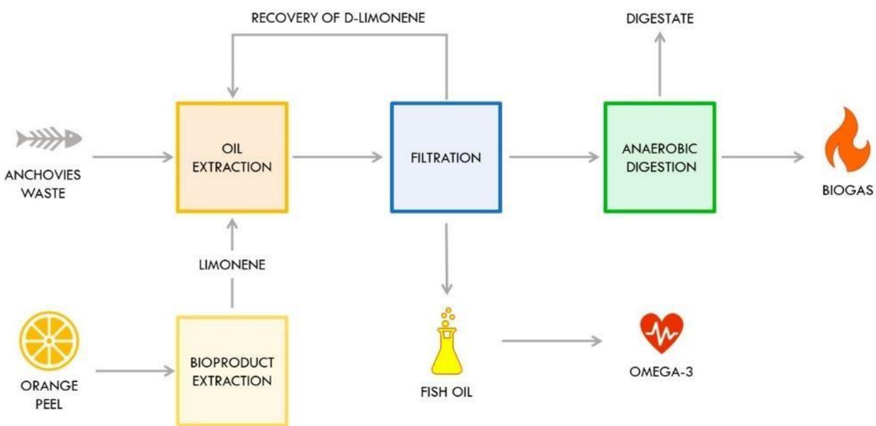


Figure 3.1 Biorefinery scheme for the full valorisation of anchovy waste.

In fact, GAC demonstrated multiple benefits for anaerobic digestion, such as adsorption of toxic substances, triggering direct interspecies electron transfer (DIET) [37][38][39][40][41][42][43].

3.1.1 Fish oil extraction process with *d*-limonene

The fish oil extraction process was carried out following the procedure previously reported [18]. After the homogenization of anchovy residues (300 g) by a blender is carried out, they are mixed with a first aliquot of *d*-limonene (150 g) refrigerated at 4 ◦ C. The so-obtained semi-solid slurry is added to a second aliquot of cold *d*-limonene (150 g) into a glass beaker sealed with aluminium foil and further coated with parafilm. The mixture is then magnetically stirred at 700 rpm for 24 h at room temperature, with the fish oil obtained by rotavaporing the supernatant at 90 ◦ C (pressure: 40

mbar). The by-product of the process is anchovy sludge (AS), still rich in *d*-limonene.

While Solid Anchovy Sludge (SAS in the following) is a treated AS with a reduced *d*-limonene quantity: AS is then dried in oven at 70 °C for 3 days and, before use, crushed in a ceramic mortar. A first BMP test was conducted with SAS, a substrate with a minor quantity of *d*-limonene, then AS was used as substrate for a BMP test and a semi-continuos experiment.

3.1.2 Substrates and inoculum characterization

A first BMP test was conducted with SAS, a substrate with a minor quantity of *d*-limonene, then AS was used as substrate for a BMP test and a semi-continuos experiment.

The morphological and elemental composition information of the solid anchovy sludge (SAS), used in the first BMP test, was achieved with a Phenom Pro-X scanning electron microscope (SEM) equipped with an energy-dispersive X-ray (EDX) using the SEM–EDX technique [44].

Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were performed on a Netzsch instrument under a helium atmosphere from room temperature to 1000 °C at a heating rate of 10 °C/min.

The analysis of the residual limonene present in the substrate was carried out by mixing 0.3 g of SAS/AS with 3 mL of a toluene solution (as an internal standard) in cyclohexane (0.1 M) for 6 hours [45]. The liquid suspension was then filtered and injected into an offline GC-FID (Agilent 6890 N) equipped with a CP-WAX 52CB column (60 m, i.d. 0.53 mm) according to the analytical procedure reported previously [46][47].

The biochemical methane potential (BMP) consists of measuring volumes of biogas and methane, produced by batches loaded with inoculum, organic substrate (SAS in this case), diluting water and nutrient solutions. The inoculum used in this experiment was a liquid digestate coming from a full-scale anaerobic digestion plant that treats manure and various residues from the agro-industry, located in the Reggio Calabria province (Italy). After the collection, inoculum was sieved to remove undigested materials (e.g., straw) and then stored at 35 °C for few days until the test started.

Both the inoculum and the substrate were characterized in terms of pH, total solids (TS) and volatile solids (VS) according to standard methods [48] before the test. Moreover, only the SAS, chemical oxygen demand (COD) and carbon to nitrogen (C/N) ratio were measured by photometric determination (WTW Photolab S12) using specific pre-dosed cuvettes (COD Cell Test 114555) and the elemental analyzer TOC-LCSH (Shimadzu, Kyoto - Japan), respectively.

Anchovy Sludge (AS), directly derived from fish oil extraction, and a co-substrate, mimicking fruit and vegetable market waste (MW), and composed of 49.0% w/w of potatoes, 44.4% w/w of apples, and 6.6% w/w of carrots, was also used for another BMP test and a semi-continuous experiment. Both substrates were characterized by measuring the total and volatile solids, pH, and carbon to nitrogen ratio (C/N), while *d*-limonene content was determined only in the AS.-The inoculum used in batch and semi-continuous experiments was a digestate coming from previous experiments. It was characterized by measuring total and volatile solids and pH evaluated according to standard methods [48]. Table 3.1 reports the main characteristics of substrates and inocula.

Table 3.1 Substrates and inocula characteristics.

	Anchovy Sludge	**Market Waste**	**Inoculum (Batch Tests)**	**Inoculum (Semi-Continuous t.)**
TS [%]	20.1	19.4	3.9	3.1
VS [%TS]	66.7	93.3	66.6	66.7
pH	6.85	5.26	8.13	8.04
C/N	3.4	36.3	-	-
d-limonene [g/g]	0.125 [1] 0.160 [2]	-	-	-

[1] A sample of AS used for batch and semi-continuous tests (days 1–56); [2] A sample of AS used for semi-continuous tests (days 57–80)

3.1.3 Evaluation of the Biochemical Methane Potential of SAS

The BMP test was carried out according to a method that was extensively used in previous studies (e.g., [49]) and in compliance with the UNI/TS 11703:2018 Italian norm and standardized protocols [50]. The method involves the use of 1.1 L glass bottles (WTWGermany) as hermetically sealed batches. Each of them has two side necks equipped with perforable septa for biogas collection and a main central neck closed by a stopper. The bottles were placed into a thermostatic cabinet at 35 ± 0.5 °C (mesophilic conditions) and kept under continuous mixing by a magnetic stirrer. Periodically, the generated biogas was withdrawn from the batches, using a 100 mL syringe, and transferred into an alkaline trap (NaOH solution, 3 M) where carbon dioxide was absorbed while the methane caused an increase of the pressure in the trap, which resulted in a displacement of an equal volume of the solution measured in a graduated cylinder. In this way, the percentage of methane in the generated biogas was evaluated.

The BMP test also included blank assays (in duplicates) that were only filled with inoculum in order to measure the non-specific methane production, the internal controls (in duplicates) fed with α-cellulose (CAS 9004-34-6, Sigma-Aldrich) for the validation of the process as required by the UNI/TS 11703:2018 norm, and lastly, the batches (in triplicates) loaded with SAS. Likewise, the inoculum and substrate cellulose were also characterized.

In each batch, the volumes of inoculum, diluting water and nutrient solutions, prescribed by the aforementioned norm in order to supply macro- and micronutrients for the bacteria metabolism, were mixed up to a working volume of 350 mL. The solutions were designed as A, B and C, and contained KH_2PO_4, $Na_2HPO_4 \cdot 12H_2O$, NH_4Cl (A, 5% of the total working volume), $CaCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$ (B, 5% of the total working volume) and $MnCl_2 \cdot 4H_2O$, H_3BO_3, $ZnCl_2$, $CuCl_2$, $Na_2MoO_4 \cdot 2H_2O$, $CoCl_2 \cdot 6H2O$, $NiCl_2 \cdot 6H_2O$, Na_2SeO_3 (C, 1% of the total working volume). The amounts of cellulose and SAS (2.1 and 2.7 g, respectively) were added in order to reach a substrate to inoculum ratio (on a VS basis) equal to 0.3. Finally, the solids concentration in the batches did not exceed 50 $g_{TS} \cdot L^{-1}$, as the regulations recommend. Before any test started, the pH of each batch mixture was measured (Table 2.2).

The BMP values of the internal controls and substrate-fed batches were expressed as the volume of produced methane gas under normal conditions (273.15 K and 101.33 kPa) per mass of VS added ($mL_{CH4} \cdot g_{VS}^{-1}$) and determined by subtracting the average methane production of the blanks (inoculum). In accordance with the regulations, the BMP test was stopped when the daily methane production was lower than 1% of the total cumulated methane volume, determined starting from the test beginning. This evidence emerged on day 34.

The net specific cumulative methane production of the batches fed with SAS were modeled using the modified Gompertz equation, Equation (1) [51]:

(Eq. 1) $$B = P \cdot exp\left\{-exp\left[\frac{R_m}{P} \cdot (\lambda - t) + 1\right]\right\}$$

where B [$mL \cdot g_{VS}^{-1}$] stands for the specific methane production at time t (d), P [$mL \cdot g_{VS}^{-1}$] stands for the methane production at time $t = \infty$, R_m [$mL \cdot g_{VS}^{-1} \cdot d^{-1}$] stands for the maximum methane production rate and λ [d] stands for the lag phase duration. P, R_m and λ were determined by minimizing the sum of square errors between the model and the experimental average values through the Excel tool "Solver".

Table 3.2 Experimental reactor settings.

Substrate	Batch	Substrate [g]	TS Mix	pH Mix
-(blank)	1	-	2.5%	7.38
	2			7.42
Cellulose (control)	3	2.1	3.1%	7.50
	4			7.47
SAS	5	2.7	3.3%	7.39
	6			7.30
	7			7.41

At the end of each test, the digestates were analyzed to determine the pH, TS and VS [48]. Furthermore, resulting from the centrifugation (10.000 rpm per 10 min), the total ammoniacal nitrogen (TAN), the Cl^- content using pre-dosed cuvettes

(Ammonium Cell Test 114,559 and Chloride Cell Test 114730, respectively) and the photometric determination (WTW Photolab S12) in the liquid fraction, the total volatile fatty acids (VFAs) concentration and the volatile organic acids/buffering capacity (FOS/TAC) ratio were determined. In particular, the latter two parameters were determined through a four-point titration method [52] consisting of titrating 20 mL of centrifuged digestate up to pH values of 5.0, 4.4, 4.3 and 4.0 with a 0.1 N sulphuric acid solution. The parameters were calculated by using Equations (2) and (3) [52][53]:

$$VFAs = \left[131340 \cdot \left(V_{pH_{4.0}} - V_{pH_{5.0}}\right) \cdot \frac{N_{H_2SO_4}}{V_{sample}}\right] - \left[3.08 \cdot V_{pH_{4.3}} \cdot \frac{N_{H_2SO_4}}{V_{sample}} \cdot 1000\right] - 10.9$$

(Eq. 2)

$$FOS/TAC = \frac{\left[\left(V_{pH_{4.4}} \cdot 1.66\right) - 0.15\right] \cdot 500}{V_{pH_{5.0}} \cdot 250}$$

(Eq. 3)

where *VFAs* and *FOS* are reported as the acetic acid equivalent ($mg_{HAC} \cdot L{-}1$) and *TAC* as the lime equivalent ($mg_{CaCO}3 \cdot L{-}1$); and $V_{pH5.0}$, $V_{pH4.3}$, $V_{pH4.4}$ and $V_{pH4.0}$ stand for the volumes recorded for acid consumption corresponding to the respective pH values, while N_{H2S04} and V_{sample} represent the normality of the acid solution (0.1) and the volume in mL of the sample (20), respectively.

3.1.4 Evaluation of the Biochemical Methane Potential of AS, MW and a mixture of both

BMP tests (Table 3.3) were performed in triplicate under mesophilic conditions using a self-developed method [54][55], basically compliant with UNI/TS 11703:2018 (the Italian standard procedure for BMP tests). Tests were performed using glass bottles (1.1 L volume) placed in a thermostatic cabinet at 35 ± 0.5 ◦ C and mixed by using a magnetic stirrer. The inoculum was mixed with the substrate (substrate to inoculum ratio in terms of vs. was set equal to 0.3) and nutrient solutions (prepared and dosed according to UNI/TS 11703:2018). BMP tests were performed to evaluate the potential methane production from AS, MW, and from a mixture of both. The mixture was prepared with the aim to obtain a C/N in the substrate equal to 25, which can be considered a well-balanced value. To obtain the desired C/N, a proportion of 1:19 (5–95) on the TS basis of AS and MW, respectively, was necessary.

In addition to BMP bottles, blanks (containing inoculum and a nutrients solution, used to assess a non-specific biomethane production) and cellulose-fed reactors (used as control) were also prepared. About three times per week, the biogas produced was transferred in a bottle filled with a NaOH solution (3M) for CO_2 adsorption, and the methane amount in the produced biogas was then measured by an eudiometer (a water displacement method). The pH was measured at the beginning of the tests, while TS, VS, COD, ammonium ion and chloride concentration, VFAs, and FOS/TAC were measured at the end of them. VFAs and FOS/TAC allow verification of the stability of the digestion process since, when a high level of them is registered or when they tend to increase over time, an unbalance of the process, due to an overloading or an inhibition of the methanogenesis, is possible [56][57]. TS, VS, and pH were measured using standard methods [48]; COD, ammonium ion, and chloride concentration were evaluated thanks to a photometric method (Photometer WTW Photolab S12 and appropriate pre-dosed cuvettes), whereas VFAs were determined through a three-point titration method, and then the FOS/TA C was calculated [56][57].

Table 3.3 BMP design of experiments.

Substrate	**Market Waste (MW)**	**Anchovy Sludge (AS)**	**Mix (95% MW + 5% AS)**
pH	8.1	8.1	8.1
C/N	36.31	3.41	24.73
$gVS_{substrate}/gVS_{inoculum}$	0.30	0.30	0.30
TS [g]	3.35	4.69	3.40
TS at the beginning of the experiment	3.17%	3.39%	3.18%

3.1.5 Semi-continuous anaerobic digestion of MW and a mixture of MW and AS

Semi-continuous experiments were carried out with the aim to reproduce more precisely, at a laboratory scale, the digestion process. A Bioprocess Control Bioreactor simulator system equipped with 4 continuously stirred tank reactors (glass, working volume 1.8 L) placed in a thermostatic water bath (set at an operating temperature of 35 ◦ C) was used. This system allows the feeding and discharge of the reactors and the measurement of the produced biomethane by a patented system based on water/gas displacement. The hydraulic retention time was set equal to 20 days, while the organic loading rate, initially set at 2.0 $g_{VS}\cdot L^{-1}\cdot day^{-1}$ during the start-up (days 0–38), was reduced before the beginning of the regime phase (days 39–83—more than 2·HRT), since a severe overloading was evident in all the reactors.

In order to accelerate the recovery of the reactors, the supplementation of new inoculum was also necessary in some experiments and, for this reason, only data recorded in the regime phase are presented and discussed. The reactors were fed three times per week; the pH was measured during each feeding/discharge and $NaHCO_3$ was added if the measure value was <6.7.

A composite weekly sample was prepared for analyses of TS, VS, COD, ammonium ion concentration, VFAs, and FOS/TAC using the same methods mentioned for batch tests. During the operation, due to the change of the AS and to the subsequent increase of the *d*-limonene, signs of inhibition of the process were evident. For this reason, as already mentioned, 10 g·L^{-1} of granular activated carbon (CARBOSORB 2040, 20 × 40 mesh; Comelt srl, Milan, Italy) were added in reactor 3; this concentration was then kept constant until the end of the test. The test was stopped after 83 days due to the unavailability of the lab for the following weeks due to reasons independent of the will of the authors.

Table 3.4 summarizes the main characteristics of the reactors during the regime phase (days 39–83).

Table 3.4. Semi-continuous experiments.

	Reactor 1	**Reactor 2**	**Reactor 3**	**Reactor 4**
Reinoculation (end of start-up phase)	YES	YES	NO	YES
Loading (regime phase) [gVS·L^{-1}·day^{-1}]	1	0.5	1	0.5
Market Waste (TS basis)	100%	100%	95%	95%
Anchovy Sludge (TS basis)	-	-	5%	5%
C/N substrate	36.3	36.3	24.7	24.7
Substrate addition [g·d^{-1}] (regime phase)	10.00	5.00	10.10	5.05

	Reactor 1	Reactor 2	Reactor 3	Reactor 4
Expected regime *d*-limonene conc. [mg·L^{-1}]	-	-	680 [1] 870 [2]	340 [1] 436 [2]
Addition of GAC—10 g·L^{-1} (days)	-	-	74–83	-

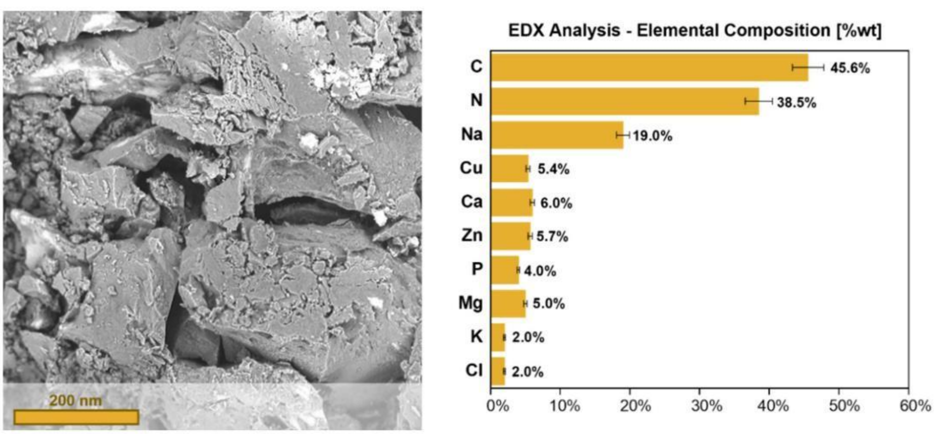
[1] Anchovy Sludge 1—days 1–56; [2] Anchovy Sludge 2—days 57–83.

3.2 Experimental section

3.2.1 BMP tests on SAS: results

The SEM images clearly show the presence of an irregular and amorphous surface in the SAS (Figure 3.2).

Figure 3.2 The scanning electron microscopy-energy dispersive X-ray (SEM-EDX) analysis of the solid anchovy sludge.



The EDX analysis revealed the predominant presence of carbon and nitrogen. Other mineral elements including potassium, calcium, magnesium, zinc and copper were also detected. The absence of toxic and/or heavy metal contaminants (such as lead, mercury and cadmium) in the sample is worth noting [58]. This is probably due to the short life span of the small pelagic species present in the Mediterranean sea. The thermal properties of SAS were determined by TGA and DTA analysis (Figure 3). The first degradation step (25–200 °C), corresponding to a weight loss of about 10%, can be attributed to the residual water and *d*-limonene present in the sludge. The next degradation step (200–500 °C), was probably due to degradation of organic materials and proteins, while the last weight loss at a max temperature of about 560 °C was due to combustion of the remained carbon and inorganic phase (including bones and scales) [59]. The characterization of the inoculum and substrates is summarized in Table 3.5. The solids content of the SAS was quite high due to the drying carried out after the extraction process.

The organic matter content, measured as VS and COD, was lower than that expected from other studies, probably because of the extraction process. Furthermore, the high protein content of the fish resulted in a very low C/N ratio. Lastly, since the *d*-limonene was used as the extraction solvent, its residual presence in the substrate was detected.

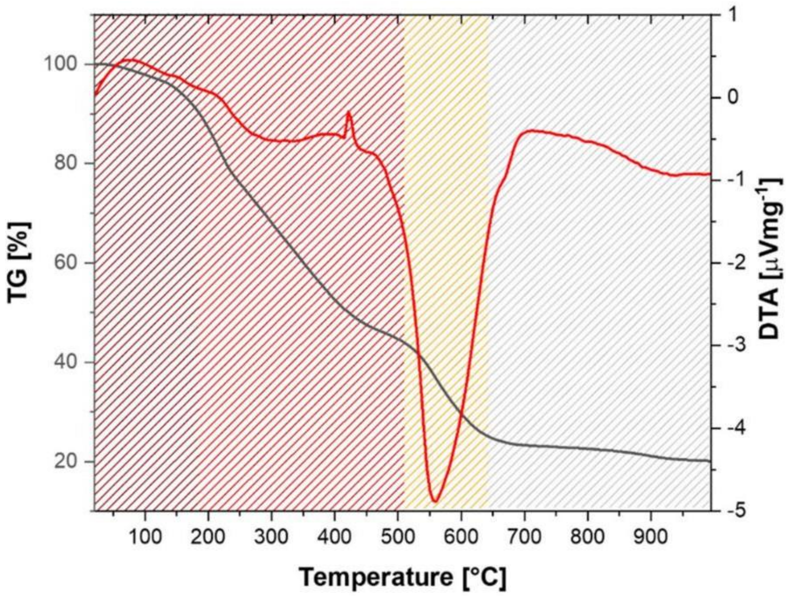


Figure 3.3 Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) analysis of the solid anchovy sludge.

Table 3.5 Characterization of inoculum, cellulose and solid anchovy sludge (SAS).

	pH	TS [%]	VS [$\%_{TS}$]	COD [$mg_{O2}\cdot g_{TS}^{-1}$]	C/N	*d*-Limonene [$mg\cdot g_{TS}^{-1}$]
Inoculum	7.50	5.0 ± 0.10	76.3 ± 0.18	-	-	-
Cellulose	-	95.6	100	1185 *	-	-
SAS	6.30	98.0 ± 0.15	77.1 ± 0.27	918.3	4	5

* Estimated from the stoichiometry.

Biogas productions, BMP values and average methane contents for the internal control and SAS-fed batches are summarized in Table 3.6. The average cumulated biogas and methane production trends of the three replicates fed with the tested substrate are depicted in Figure 3.4.

Table 3.6 Biogas and methane final production and methane content in biogas volumes of control and SAS-fed batches.

Substrate	Batch	Biogas [mL·g_{VS}^{-1}]	Average [mL·g_{VS}^{-1}]	BMP [mL_{CH4}·g_{VS}^{-1}]	Average [mL_{CH4}·g_{VS}^{-1}]	Average Methane Content
Cellulose (control)	3	603.4	598.3	396.5	390.0	68%
	4	593.3		382.6		65%
SAS	5	406.4	378.5	296.1	278.0	72%
	6	381.7		281.3		73%
	7	347.4		256.4		73%

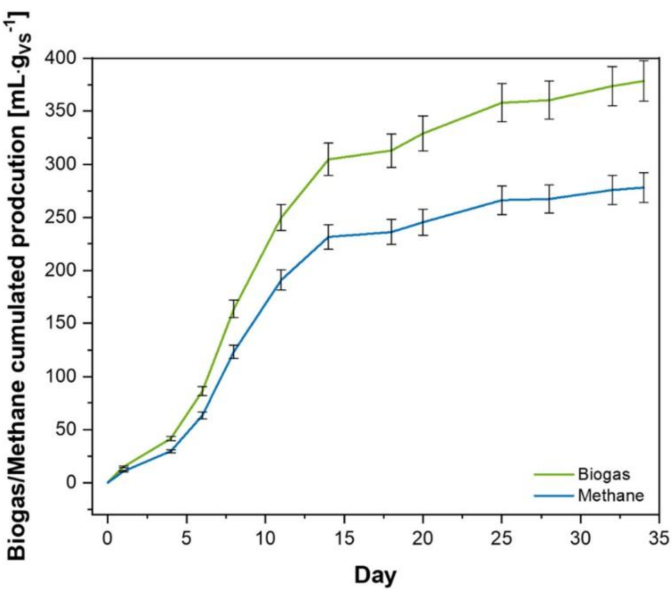


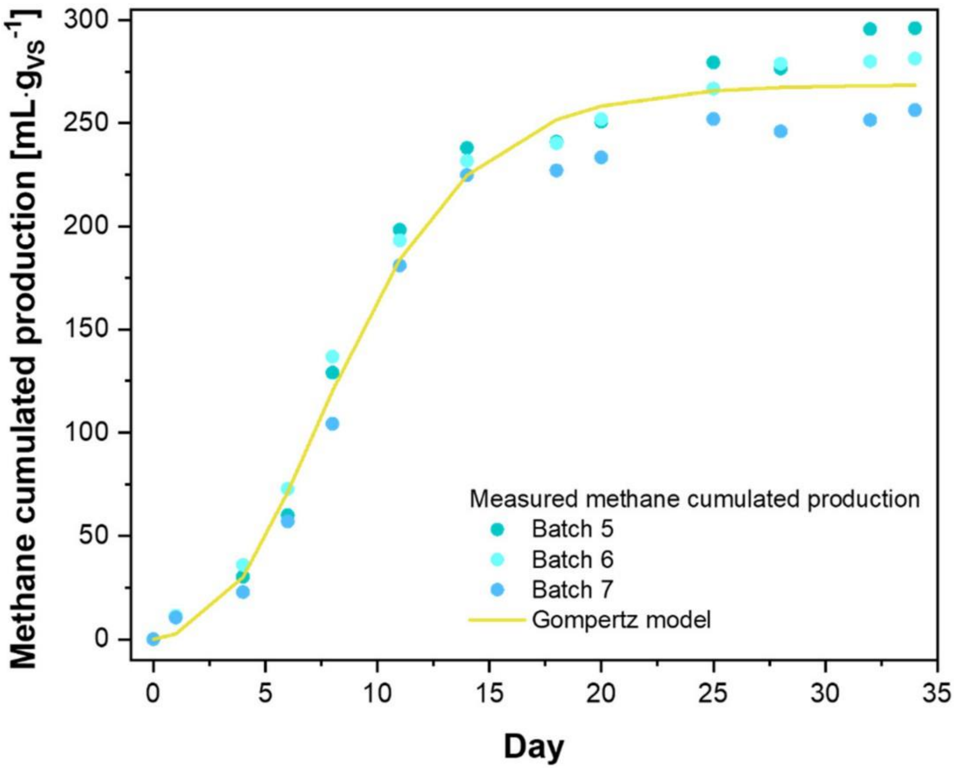
Figure 3.4 Cumulated biogas and methane production trends of batches fed with SAS.

The average cumulated methane production of the substrate batches was modeled with the modified Gompertz equation (Figure 3.5), and the respective kinetic parameters calculated are summarized in Table 3.7.

The BMP values of the internal controls met the UNI/TS 11703:2018 requirements of 325 ± 25% $mLCH_4 \cdot g_{VS}^{-1}$ and a difference lower than 10% was found. The results support the necessary validation of the BMP test. With regard to the batches fed with SAS, batch 5 showed a slightly higher methane production than the other replicates. However, it is notable that final BMP values of the three batches were quite close to each other, indicating that the respective anaerobic processes were even. Indeed, when evaluating the processes in terms of biogas/methane generation, the digestions also performed similarly in the three replicates. In fact, after a small acclimatation period of a few days, the gas production increased faster up to the 14th day. Then, the batches continued to generate biogas/methane, albeit with a slower rate, until the 34th day when the test was stopped.

Moreover, the practically identical methane contents in the biogas volumes confirmed that the anaerobic digestion processes of the three replicates proceeded with similar trends.

The Gompertz interpolating model fits the average of the experimental measurements of the three replicates ($r^2 > 0.99$) well. The ultimate methane production at time ∞ was predicted to be



268.7 mLCH_4·g_{VS}^{-1} in good agreement, although it was slightly lower with the average BMP value at the end of the test.

Figure 3.5 Simulation of cumulated methane production by the Gompertz model.

Table 3.7 Kinetic parameters of the Gompertz equation.

P [mL·g_{VS}^{-1}]	R_m [mL·g_{VS}^{-1}·d^{-1}]	λ [d]	r^2
268.7	24.8	3.145	0.997

The lag phase duration, found using the Gompertz model of about 3 days, was consistent with the initial low biogas/methane production depicted in Figure 3.5. Lastly, in Table 3.8 the chemical characteristics of the residual digestates are summarized. In contrast with the blanks and controls, the pH values of the mixtures of the SAS-fed reactors at the end of the experiment did not vary significantly compared to those measured at the beginning of the test (Table 3.8).

Table 3.8 Characterization of solid anchovy sludge digestate.

Property	Blank	Cellulose	SAS
pH	7.1 ± 0.06	7.0 ± 0.01	7.3 ± 0.00
TS	2.4 ± 0.03%	2.5 ± 0.02%	2.7 ± 0.01%
VS	71.2 ± 0.19%	72.1 ± 0.18%	69.3 ± 0.57%
TAN [$mg·L^{-1}$]	234 ± 8.8	173 ± 8.0	697 ± 20.3
Cl^- [$mg·L^{-1}$]	1263 ± 17.5	1105 ± 55.0	1297 ± 107.3
VFA [$mg_{HAC}·L^{-1}$]	297.3 ± 14.11	290.4 ± 21.04	411.1 ± 28.09
FOS/TAC [$g_{HAC}·g_{CaCO3}^{-1}$]	0.08 ± 0.001	0.09 ± 0.010	0.09 ± 0.008

In terms of the TS and VS content, no difference among the different assays was detected. Considering the initial total solids content of each reactor mixture, it can be noticed that in the control and tested substrate-fed reactors the solid matter was consumed by the microbial process, while in the blanks, the solid content changed to a lower extent. The ammonium content was clearly higher in the residual digestates of the reactors loaded with SAS than in the others. This was predictable since the tested substrate showed a low C/N ratio which, in the digestion process, resulted in ammonium accumulation.

On the other hand, the determination of the Cl^- concentration did not exhibit differences among the different digestates. The total VFAs content was slightly higher in the residual digestates of the tested substrate-fed reactors than in the other assays, while for the FOS/TAC ratios, no differences among the different assays were observed. The very low calculated FOS/TAC ratios suggest that all the organic matter was consumed by microorganisms for each reactor. The SAS methane yield was consistent with the range observed for fish waste anaerobic digestion (200–900 $mLCH_4 \cdot g_{VS}^{-1}$) reported by Ivanovs et al. [30]. Comparing only studies on the anaerobic digestion of fish oil extraction residues, the BMP of our test was lower, by far, than 742 $mLCH_4 \cdot g_{VS}^{-1}$ (the residue of salmon heads enzymatically hydrolyzed in Nges et al. [27]) and 426 $mLCH_4 \cdot g_{VS}^{-1}$ (residue of carp viscera thermos-mechanically pretreated in Bucker et al. [28]). In these studies, as in the present one, nitrogen inhibition was not observed despite the low C/N ratios. This was probably due to the positive inoculum influence. First of all, inoculum can contribute to balancing the substrate nitrogen content, as noticed by Vivekanand et al. [60] and confirmed by Bucker et al. [28]. In the aforementioned studies, the methane yields of fish oil extraction residues were about 10% and 20% lower than those determined by raw fish waste digestion (828 and 541 mLCH4·gVS−1 in Nges et al. [27] and Bucker et al. [28], respectively). This suggests that oil extraction does not severely affect the potential use of fish waste as the substrate in anaerobic digestion.

However, further research could be carried out in order to investigate other possible pre-treatments of both fish waste and oil extraction residues. Process conditions very similar to ours were reported by Eiroa et al. [26]. In their study on the anaerobic digestion of four different fish wastes, the methane yield and final TAN content were 285 $mLCH_4 \cdot g_{VS}^{-1}$ and 728 $mg \cdot L^{-1}$ (both on average), respectively. Inhibition was signalized by Morales-Polo et al. [29], where the anaerobic digestion of the anchovy waste generated only 4.6 $mLCH_4 \cdot g_{VS}^{-1}$ because of a distinct ammoniacal nitrogen accumulation (TAN concentration of 6.13 $g \cdot L^{-1}$). For this reason, it is possible that co-digestion with an additional substrate that has a higher C/N would be beneficial, as already demonstrated for other N-rich substrates (e.g., manure, slaughterhouse waste) [61][62].

Moreover, the residual *d*-limonene concentration in the anaerobic mixture was found to be around 40 $mg \cdot L^{-1}$, well below the level tolerable for a stable AD [32]. However, the initial slow methane production and the peculiar production trend could be due to the adaptation of anaerobic microorganisms to the *d*-limonene presence [34].

3.2.2 Experiments with AS, MW and a mixture of both: results

3.2.2.1 BMP tests on AS, MW and a mixture of both: results

The full valorization of anchovy fillet processing waste requires converting the residual sludge after the extraction of fish oil rich in omega-3 and vitamin D_3. In the previous report, we used the aforementioned solid anchovy sludge (SAS) as a biobased substrate in anaerobic digestion aimed at producing biogas. A good methane yield of about 280 $mL_{CH4}\cdot g_{VS}^{-1}$ was obtained. The overall process was very stable, thus making the solid anchovy sludge a suitable substrate for co-digestion with other biomass wastes and residues. Indeed, taking into account the high nitrogen content of the fish waste, we also demonstrated that an optimal carbon to nitrogen ratio (C/N) is advisable in order to maximize biogas production.

Methane production during the BMP test was regular for batches fed with the MW or the mixture between the former and AS (Figure 3.6 and Table 3.9). Final BMP values were very similar: 421

± 13 $NmL \cdot g_{VS}^{-1}$ for MW and 420 ± 23 $NmL \cdot g_{VS}^{-1}$ for the mixture respectively; the very low standard deviation witnesses the uniformity of the production among batches. Moreover, results confirm those reported in the scientific literature for similar substrates [63][64], corroborating that MW is an excellent substrate for anaerobic digestion. Notably, due to the supply of nitrogen from the inoculum, the benefit of optimizing the C/N ration by adding the AS is not evident in batch tests.

The results relative to batches fed uniquely with AS are completely different, even if the difference was expected. At the end of the experiment, their production was, on average, lower than that of the blank (inoculum); in this case, the standard deviation was also very limited, thus confirming the consistency of the results. The most likely reason for the observed behaviour is due to the presence of *d*-limonene in the substrate. In fact, the initial concentration of the latter at the beginning of the experiment was of about 4870 $mg \cdot L^{-1}$ and therefore well above the level severely inhibiting anaerobic digestion [36].

The analyses on digestate at the end of the experiments (Table 3.8) further confirm these trends: while the reactors fed with MW and with the mixture perform similarly (and similarly to the positive control fed with cellulose data; not shown) in terms of residual COD, ammonium ion and chloride concentration, and VFA, the batches fed with AS only present a higher residual COD (+63%) and a high concentration of VFA (+510%). The latter indicates that the conversion of the substrate to VFA occurs, but the high concentration of *d*-limonene severely affects the methanogens, triggering the accumulation of VFA up to toxic values [65][66][67]. The results of the batch tests indicate that AS, even in the presence of quite high amounts of *d*-limonene, is potentially a good substrate for co-digestion with carbonaceous feedstocks.

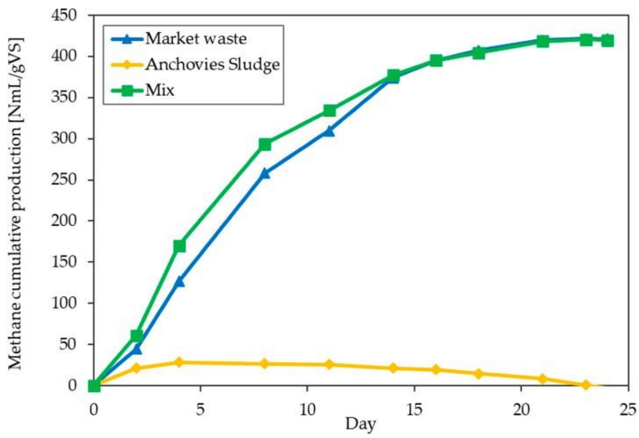


Figure 3.6 Methane cumulative production during BMP tests.

Table 3.9 Analyses of digestate from BMP experiments.

	Market Waste	**Anchovy Sludge**	**Mix (Market w. + Anch. Sludge)**
pH	7.6 ± 0.00	7.6 ± 0.06	7.5 ± 0.01
COD [mg/L]	7008 ± 398	11470 ± 130	7073 ± 385

	Market Waste	**Anchovy Sludge**	**Mix (Market w. + Anch. Sludge)**
ammonium ion [mg/L]	1435 ± 60	1861 ± 61	1411 ± 39
chloride [mg/L]	1280 ± 93	1563 ± 105	1363 ± 274
VFA [mg/L]	550 ± 156	3662 ± 69	651 ± 129
FOS/TAC	0.11 ± 0.03	0.4 ± 0.02	0.12 0.02

3.2.2.2 Semi-continuous experiments with MW and a mixture of MW and AS

In the initial part of the regime phase (Figure 3.7b), methane production seems to be linked only to the applied organic loading. In fact, reactors 1 and 3, and 2 and 4, respectively, behave similarly. This behavior was confirmed for reactors 2 and 4 (low loading) until the end of the experiment, while reactors 1 and 3 present a different production pattern. Reactor 1 and 3's productions slowed gradually since about day 60 for the latter; this tendency was more pronounced, but a sudden recovery was also evident from about day 75 of the total operation.

For the first 20 days of regime phase (1·HRT) the average yield (Figure 3.7b) was similar for reactors 1 and 3, and 2 and 4, respectively; it was equal to about 0.2 $NL \cdot gVS_{added}^{-1}$ for reactors 1 and 3 and to about 0.25 $NL \cdot gVS_{added}^{-1}$ for reactors 2 and 4, respectively. Then a continuous decrease was evident for reactor 1 that reached a value of about 0.17 $NL \cdot gVS_{added}^{-1}$ in the last days of the experiment. On the contrary, after a sharper decrease until day 75, first stabilization and then a slight increase was registered; in the last days of operation the yield of reactor 3 surpassed that of reactor 1. pH (Figure 3.7c) in all reactors during the regime phase was close to 7, seldom needing $NaHCO_3$ addition to increase the buffering capacity. A marked tendency toward a reduction is evident in reactor 3 after the beginning of the feeding of the new sludge.

TS (data not displayed) and VS concentrations (Figure 3.7d) were stable during the regime phase; TS were slightly higher for reactors 1 and 3 (loading 1 $gVS \cdot L^{-1} \cdot day^{-1}$).

The COD concentration (Figure 3.7c), except for a few spikes (e.g., week 9—reactor 2), is quite low when the process is stable and tends to increase when the process is inhibited (reactors 1 and 4, since weeks 9–10).

The ammonium ion concentration (Figure 3.7f) displays a tendency to decrease and reaches very low values (it is practically absent) in reactor 1 since week 4 of operation and in reactor 2 since week 6.

The VFA concentration and FOS/TAC (Figure 3.7g,h) are two important process stability indicators, they are conveniently low for reactors 2 and 4, while they display a tendency to increase since week 10 for reactors 1 and 3. In reactor 3, after the beginning of GAC addition, VFA suddenly decreases and the FOS/TAC stabilizes.

It is worth pointing out that the reactors during the start-up phase, as mentioned above, suffered from a severe overloading that, however, did not seem to significantly influence the regime phase, indicating a good recovery. The fact that differences among reactors operating at the same loading, although using different substrates (either MW only or a mix between this and AS), were not detected during the initial regime phase can be attributed to two main factors: (i) the supply of nitrogen in reactors 1 and 2 (fed only with MW) was linked to reinoculation (see Table 3.9), operating to re-establish the process after the already mentioned overloading, and (ii) the adaptation to *d*-limonene during the start-up phase of the microbial population of reactor 3 (the one with the highest *d*-limonene loading, as reported in Table 3.9) was the only one not needing reinoculation.

The reasons for the reduction of the methane production after about 1·HRT in the regime phase in reactors 1 and 3 can be attributed to two different factors. For reactor 1, the reduction of nitrogen below tolerable limits is evident (see Figure 3.7f) and this supports the idea that C/N optimization is essential for stable anaerobic digestion. Indeed, adequate nitrogen presence in digesters must be ensured since it is involved in the fundamental activities of microbial metabolism (synthesis of proteins, enzymes, ribonucleic acid (RNA), and deoxyribonucleic (DNA)) [68]. Thus, the lack of nitrogen could have affected the anaerobic bacteria's metabolism, eventually causing process failure.

For reactor 3, besides the nitrogen reduction also detected in this case, the feeding of the new AS (see Table 3.9) most probably increases the *d*-limonene concentration up to intolerable levels and triggers an inhibition of the methanogenesis, as the sharp increase in VFA concentration and in FOS/TAC and the significant pH reduction both witness. The supplementation of GAC (10 $g \cdot L^{-1}$) since day 74 and until the end of the experiment causes an almost immediate recovery of the reactor, with a sharp increase in methane production since day 77 and until the end of the experiment. These results confirm previous research [37][43] on the potential of this material in sustaining the anaerobic digestion of *d*-limonene containing substrates. Also, if the experiment, as already mentioned, was forcedly terminated after about 10 days since the beginning of the GAC addition, its effect on the process stabilization (probably mainly linked to the adsorption of d-limonene) are evident. It is interesting to note that in reactor 4, with a potential *d*-limonene concentration close to 450 $mg \cdot L^{-1}$, the process does not demonstrate signs of disruption, confirming the potential of the microbial community to adapt to *d*-limonene and therefore the importance of an optimized loading when using AS for the co-digestion with a carbonaceous substrate. This optimization should aim to slowly increase the quantity fed to the reactor to keep the *d*-limonene concentration at a tolerable level. On the other hand, two other factors are very important and worth noting: (i) optimization of the recovery of *d*-limonene during the extraction of the fish oil would be beneficial for the entire biorefinery scheme, and (ii) GAC is confirmed as a powerful additive in the anaerobic digestion of substrates containing *d*-limonene.

The yield of the process was lower than expected, compared to the BMP value for the co-digestion of MW and AS (420 ± 23 $NmL \cdot gVS^{-1}$) with respect to batch tests; the reduction is evident and in the order of 40% for the low loaded reactors and 50% for the others. Furthermore, this reaction is more pronounced than the 10–30% often reported in the scientific literature for batch and semi-continuous tests on the same substrate [69][70][71]. Since only reactors also fed with MW display similar behavior, this situation is most probably attributable more to the imperfect start-up of the reactors than to the use of AS a co-substrate.

This study demonstrates the potential suitability of AS as co-substrate for the anaerobic digestion of mainly carbonaceous feedstocks. However, the presence of *d*-limonene is an issue that requires proper countermeasures; the first is the optimization of the oil extraction process to reduce the residual of the solvent present in AS. In addition, the results presented here, although preliminary, demonstrate how proper adaptation and the supplementation of

GAC during anaerobic digestion can improve tolerance to *d*-limonene.

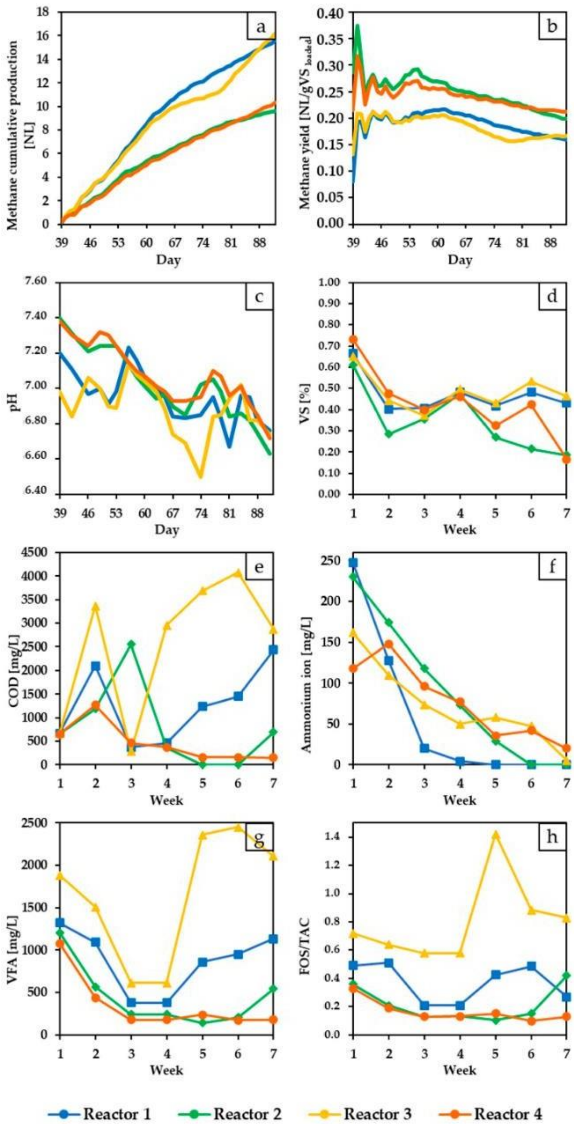


Figure 3.7 Semi-continuous experiments results: (a) methane cumulative production, (b) methane yield, (c) pH, (d) volatile solids, (e) COD, (f) ammonium ion, (g) VFA, and (h) FOS/TAC.

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Chapter 4

AnchoisFert: a new organic fertilizer from fish processing waste for sustainable agriculture

4.1 Fish oil extraction residue as organic fertilizer

Certain fish processing companies, such as tuna processing companies, have found a market for their fish biowaste by selling it to pet food producers. However, the majority of fish processing companies face the challenge of incurring costs to dispose of their processing waste. In Italy, for instance, the average tariff paid by a fish processing company for biowaste disposal in 2020 was €0.2/kg [1]. Considerable research has been dedicated in the past two decades to developing methods for converting these waste materials into valuable products, including fish oil, fish hydrolysate, collagen, chitin, chitosan, and hydroxyapatite [2]. A notable example of circular economy practices involves the extraction of fish oil from leftover anchovy biomass using a biobased solvent called limonene. This innovative process was introduced in Sicily in 2019 [3].

Approximately 50% of the whole fish is utilized for fillet production, leaving behind leftovers that are typically disposed of as biowaste at considerable economic costs. Introducing additional revenue streams for fisheries through the valorization of anchovy fillet leftovers would help mitigate the risk of overfishing, particularly when generating high-value bioproducts from this biowaste.

One such product is the innovative "AnchoisOil," a fish oil extracted exclusively from anchovy leftovers using biobased *d*-limonene as the sole solvent (Figure 4.1). This oil, rich in oleic acid, DHA, and EPA in their natural triglyceride form, also contains abundant vitamin D in the most bioavailable form (vitamin D3).

While the oily fraction extracted with limonene accounts for only 10% of the anchovy leftovers, we recognized the potential of the remaining residue, which is rich in protein, minerals, and carbon content, and explored its utilization as organic fertilizer.

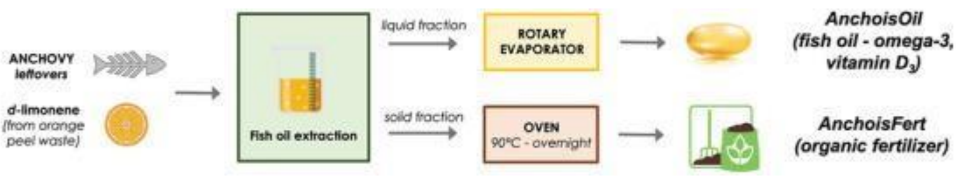

Figure 4.1 Overall upgrading process of anchovy fillet leftovers converted into AnchoisOil and AnchoisFert.

Chemical fertilizers, typically marketed in the form of powder mixtures containing nitrates, phosphates, and potassium salts, are widely employed worldwide to maximize crop yields. However, their use presents significant environmental and health challenges. This includes the accumulation of heavy metals, inorganic acids, and organic pollutants in the soil [4] and groundwater [5], as well as the depletion of organic carbon in agricultural soils.

By utilizing the extraction residue from anchovy leftovers as organic fertilizer, we aim to address these concerns and offer a more sustainable alternative to chemical fertilizers.

In addition to the environmental and health concerns associated with chemical fertilizers, their high and continuously increasing costs make them economically unviable for many farmers. Therefore, there is a strong desire to replace chemical nutrients with organic fertilizers. However, one of the main challenges with organic fertilizers is their poor performance, as their effects on crop yield are often slow and variable [6].

The development of highly effective and economically viable organic fertilizers is in high demand. Coupled with increased participation in farmer organizations and improved policy measures [7], the availability of such fertilizers could persuade farmers to transition from chemical to organic fertilization practices.

In the following, we present a significant discovery: the solid residue obtained from the extraction of anchovy fillet leftovers using biobased limonene, referred to as "AnchoisFert," has been found to be a potent fertilizer. It demonstrates the ability to promote the growth of Tropea's red onions (*Allium cepa*) significantly better than commonly used chemical and organic fertilizers. This discovery not only completes the fishing material cycle for anchovy fishing but also greatly enhances the sustainability of both anchovy fishing and agriculture.

4.1.2 Results and discussion

The chemical and physical characterization (pH, electrical conductivity, main ions, carbon, nitrogen, total flavonoid, and phenol content) of anchovy leftovers obtained after fish oil extraction with limonene, as well as nitrogen phosphorous potassium (NPK) and horse manure (HM), was conducted to assess their relevance to agriculture. The data presented in Table 4.1 is expressed in units per gram of dried weight (DW).

The residue obtained from the limonene extraction was mildly acidic, with a pH of 6.27. It exhibited poor electrical conductivity, measuring 5.9 μS cm^{-1}. However, the residue displayed a high content of carbon (40%) and nitrogen (12%), which, along with significant amounts of valued mineral nutrients, makes it highly promising as a fertilizer. Particularly noteworthy are the elevated levels of calcium (35.2 mg g^{-1}), sulfate (16.2 mg g^{-1}), magnesium (6.7 mg g^{-1}), potassium (5.5 mg g^{-1}), and phosphate (5.6 mg g^{-1}). These properties indicate the potential for its effective use as a fertilizer in agricultural applications.

Furthermore, the anchovy residue contains bioactive compounds, including total phenols and total flavonoids, known for their antioxidant activity. The total phenol content is measured at 8507 μg g^{-1}, expressed as tannic acid (TA), per gram of dried weight (DW). The total flavonoid content (TFC) is recorded as 1868 μg g^{-1}, expressed as quercetin equivalent (QE), per gram DW. These bioactive compounds contribute to the potential health benefits and antioxidant properties of the anchovy residue.

Table 4.2 provides the chemical parameters of non-fertilized soil (CTR), chemically-fertilized soil (NPK), and organically-fertilized soils with horse manure (HM) and AnchoisFert (AF). The analysis was conducted after a period of onion cultivation for three months. The data is expressed in units per gram of dried soil (DS), allowing for a comparison of the different fertilization methods and their impact on the soil composition.

The use of anchovy-based fertilizer limited the increase in electrical conductivity of the soil (measured at 357 μS cm^{-1}), which can be attributed to the residual limonene present in the anchovy leftovers after the extraction of omega-3. The soil treated with this fertilizer showed the highest content of total phenols (550 μg TAE g^{-1}DS), carbon (3.59%), and nitrogen (0.26%).

The specific inhibition of the dehydrogenase enzyme by the phenols contained in AnchoisFert, similar to the inhibition of shikimate dehydrogenase by various polyphenols [8], resulted in the lowest dehydrogenase activity (DHA) among all the soils studied, with a value of 1.48 μg TPF g^{-1} DS h^{-1} for the soil fertilized with AF. It is worth noting that the phenolics and flavonoids present in AnchoisFert have beneficial effects on soil microbial activity, as demonstrated by the highest level of total microbial activity measured indirectly using fluorescein diacetate (FDA) hydrolysis.

Table 4.1 Main chemical and physical parameters of anchovy leftovers after oil extraction with limonene

	AF	*NPK*	*HM*
Chemical parameter	Value	Value	Value
Total solids (TS)	20.1%	–	–
Volatile solids (VS)	66.7%	–	–
C	40%	–	30%
N	12%	10%	2%
C/N	3.3	–	15%
Total phenols	8507 μg TA/g DW	–	637.5
Total flavonoids	1868 μg QE/mg		549.5
pH	6.27		7.32
Electrical conductivity	5.945 μS cm^{-1}		6.834 μS cm^{-1}
Ca^{2+}	35.2 mg g^{-1}		0.4
K^{+}	5.5 mg g^{-1}	100 mg g^{-1}	2.2 mg g^{-1}
Mg^{2+}	6.7 mg g^{-1}	–	0.12

SO_4^{2-}	16.2 mg g^{-1}	–	0.6
PO_4^{3-}	5.6 mg g^{-1}	100 mg g^{-1}	3.1 mg g^{-1}
Limonene	0.140 mg g^{-1}	–	–

Table 4.2 Chemical and biochemical parameters of non-fertilized (CTR), chemically-fertilized (NPK), and organically-fertilized (HM and AF) soils after onion harvesting.

Fertilizer	pH (H_2O)	pH (KCl)	EC [µS cm^{-1}]	TPC [µg TA g^{-1} DS]	DHA [µg TPF g^{-1} DS h^{-1}]	FDA [µg fluorescein g^{-1} DS]	C [%]	N [%]	OM [%]	C/N
CTR	8.06 a)	6.98 a)	436 a)	351c)	1.66b)	17.6c)	2.52c)	0.22b)	4.33c)	11c)
NPK	8.14 a)	6.99 a)	438 a)	407b)	2.08 a)	21.8b)	3.21b)	0.20b)	5.52b)	16 a)
HM	7.97 a)	6.98 a)	391b)	380b)	1.68b)	16.9c)	3.54 a)	0.23b)	6.08 a)	15a,b)
AF	8.19 a)	6.89 a)	357c)	550 a)	1.48c)	32.6 a)	3.59 a)	0.26 a)	6.19 a)	14b)

The soil fertilized with AF exhibited a total microbial activity of 32.6 µg fluorescein g^{-1}DS, indicating a thriving microbial community in the presence of this fertilizer. For comparison, the soil fertilized with NPK and HM respectively contained 21.8 and 16.9 µg fluorescein diacetate g^{-1} DS.

In addition, soil organic matter (OM) reached its highest value of 6.19% in the soil that was fertilized with AnchoisFert. It is important to note that OM serves as an indicator of soil quality and is directly associated with the soil's ability to function as a nutrient reservoir. It also plays a crucial role in promoting biological activity, which, in turn, affects the availability of energy for microbial growth and enzyme production [9].

The analysis of cations and anions in the soils, including non-fertilized (CTR), chemically-fertilized (NPK), and organically-fertilized with horse manure (HM) and AnchoisFert (AF), was conducted after the harvest of onions at a 3-month growth stage. The results revealed significant differences among the different fertilization treatments (Table 4.3), with the data expressed in units per gram of dried soil (DS).

Table 4.3 Cations and anions in non-fertilized (CTR), chemically-fertilized (NPK), and organically-fertilized (HM and AF) soils after onion harvesting

Cation	*CTR (mg g⁻¹)*	*NPK (mg g⁻¹)*	*HM (mg g⁻¹)*	*AF (mg g⁻¹)*
NH_4^+	0.0045 ± 0.02 [a)]	0.059 ± 0.01 [a)]	0.061 ± 0.01 [a)]	0.072 ± 0.02 [a)]
K^+	1.4 ± 0.1 [b)]	1.8 ± 0.2 [a)]	1.9 ± 0.3 [a)]	1.9 ± 0.2 [a)]
Mg^{2+}	0.11 ± 0.07 [c)]	0.22 ± 0.06 [b)]	0.21 ± 0.05 [b)]	0.33 ± 0.04 [a)]
Ca^{2+}	0.29 ± 0.09 [a)]	0.21 ± 0.1 [a)]	0.34 ± 0.1 [a)]	0.44 ± 0.7 [a)]
Anion	CTR [mg g⁻¹]	NPK [mg g⁻¹]	HM [mg g⁻¹]	AF [mg g⁻¹]
F^-	0.01 ± 0.01 [a)]	0.03 ± 0.01 [a)]	0.006 ± 0.0 [b)]	0.01 ± 0.01 [a)]
Cl^-	0.14 ± 0.01 [d)]	0.4 ± 0.07 [c)]	0.7 ± 0.01 [b)]	1.2 ± 0.1 [a)]

Br^-	Nd	0.03 [a]	0.03 [a]	0.03 [a]
NO_3^-	0.09 ± 0.01 [b]	0.3 ± 0.02 [a]	0.5 ± 0.02 [a]	0.4 ± 0.02 [a]
NO_2^-	0.07 ± 0.01 [a,c]	0.07 ± 0.02 [a]	0.07 ± 0.01 [a]	0.07 ± 0.01 [a]
SO_4^{2-}	0.1 ± 0.01 [c]	3.5 ± 1.2 [a,b]	2 ± 0.3 [b]	4.1 ± 2 [a]

[a] Data expressed as mean of three replicates. Different letters indicate significant differences at $p < 0.05$.

In general, the treatment with AnchoisFert (AF) resulted in the largest increases in all measured cations compared to the other fertilizers. The soils fertilized with AnchoisFert showed a significant 16-fold increase in ammonium content, ranging from 0.45×10^{-2} to 7.2×10^{-2} mg g^{-1}. This was followed by a 13.5-fold increase in the presence of horse manure (HM), which is known to be rich in urea and ammonia.

In terms of potassium, both the AF and HM fertilizers led to a 35% increase in its content in the soil. This increase was higher compared to the 28% increase observed with the NPK chemical fertilizer, which primarily contains potassium nitrate.

The presence of the new fertilizer, AnchoisFert, resulted in a significant threefold increase in magnesium content in the soil, surpassing the twofold increase observed with the NPK fertilizer. Furthermore, a 1.5-fold increase in calcium was observed in the soil fertilized with AnchoisFert, followed by a 1.17-fold increase in the soil fertilized with horse manure (HM). These findings demonstrate the effectiveness of AnchoisFert in enriching the soil with essential cations necessary for plant growth and development.

Regarding anions, it is worth noting that the amount of fluoride remained unchanged at a very low level (0.01 mg g^{-1}) in bulbs grown in non-treated soil and soil fertilized with AnchoisFert (AF). However, when comparing the control soil to the soil fertilized with horse manure (HM), there was a 40% decrease in fluoride content (from 1×10^{-2} to 0.6×10^{-2} mg g^{-1}). Similar results were observed for nitrite, with its content remaining unchanged at 7×10^{-2} mg g^{-1} in all bulbs, regardless of whether they were fertilized with chemical or organic fertilizers.

The use of organic fertilizers led to a significant increase in the levels of chloride and nitrate ions in the bulbs of onion plants. Specifically, the chloride content increased from 0.14 mg g^{-1} to 1.2 mg g^{-1} and 0.7 mg g^{-1} with the application of AnchoisFert (AF) and horse manure (HM), respectively. The largest increase was observed in nitrate content, with a 5.5-fold increase (from 0.09 to 0.5 mg g^{-1}) in bulbs grown in soils treated with HM, followed by a 4.4-fold increase in bulbs grown in the presence of AF. These findings highlight the effectiveness of organic fertilizers in promoting higher levels of chloride and nitrate ions in plant bulbs.

It is noteworthy that the increases in chloride and nitrate ions observed in bulbs from plants grown in organically fertilized soils were greater than the 3.3-fold increase measured in bulbs from plants grown in soil treated with the NPK fertilizer, which is rich in concentrated nitrate ions.

Additionally, there was a remarkable 41-fold increase in sulfate ion content in bulbs grown in soil fertilized with AnchoisFert (AF), followed by a 35-fold increase in bulbs from soil fertilized with the chemical fertilizer NPK, which contains abundant sulfate anions.

Moving on to the plant growth parameters of red onions grown in pots for a period of 3 months (maturation time), significant differences were observed among the different fertilizers used. These include the chemical fertilizer NPK (20:10:10; 1.2 g/pot), horse manure (13 g/pot), and AnchoisFert (1.60 g/pot). For further details on these differences, please refer to Table 4.4.

Table 4.4 Growth parameters of red onion grown for 3 months in non-fertilized (CTR), chemically (NPK), horse manure (HM), and AnchoisFert (AF) fertilizer.

Parameter	*CTR*	*NPK*	*HM*	*AF*
Bulb weight [g]	82 ± 2[c)]	102 ± 5[c)]	101 ± 4[b)]	136 ± 5[a)]
Bulb diameter [cm]	3 ± 1[b)]	4 ± 1[b)]	4 ± 0.7[b)]	7 ± 1[a)]
Leaf length [cm]	42 ± 3[a)]	46 ± 2[a)]	42 ± 2[a)]	46 ± 2[a)]
Average plant height [cm]	55 ± 2[b)]	64 ± 3[a)]	63 ± 3[a)]	68 ± 2[a)]

[a)] Data expressed as mean ± standard error. Different letters indicate significant differences at $p < 0.05$.

Except for the leaf length, which remained unchanged at 46 cm, fertilization with AnchoisFert once again yielded the best results in terms of plant growth parameters. The plant height increased when comparing plants grown in non-fertilized soil to those grown in fertilized soil. However, the most significant change was observed in the fruit parameter, specifically the onion bulb.

For plants grown in soil fertilized with AnchoisFert, there was a 65% increase in bulb weight, from 82 to 136 g. Additionally, the bulb diameter increased by 133%, from 3 to 7 cm (Figure 4.2). These results highlight the positive impact of AnchoisFert on the size and quality of onion bulbs.

Treatment with the other organic fertilizer, horse manure (HM), led to increased weight and diameter of the onion bulb compared to the control group, similar to the effects observed with the NPK chemical fertilizer.

The analysis of cations and anions in red onion bulbs grown in pots for a period of 3 months (maturation time) with the different fertilizers, using the same amounts mentioned earlier, revealed significant differences (Table 4.5). The results are expressed in milligrams per gram of dried weight (DW). The data indicated a significant bioavailability of nitrogen, which is primarily present in horse manure as urea and ammonia, and in proteins in AnchoisFert. As a result, the amount of ammonium in red onion bulbs increased significantly.

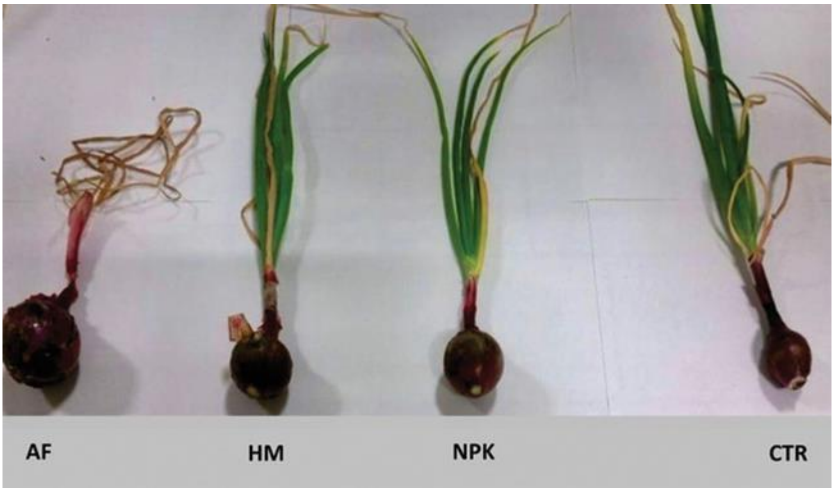

Figure 4.2 Tropea's red onion plant grown in soil fertilized with AnchoisFert (left), horse manure (HM), nitrogen:phosphorous:potassium (NPK), and in unfertilized soil (CTRL).

It went from 8.7 mg g^{-1} in non-fertilized soil to 31 mg g^{-1} and 20 mg g^{-1} in soils fertilized with horse manure (HM) and AnchoisFert (AF), respectively. These findings demonstrate the effective uptake and utilization of nitrogen by the onion bulbs when they are grown in soil enriched with these organic fertilizers.

The amount of potassium in red onions showed a slight increase of 2% and 3% when grown in soils fertilized with the organic fertilizers mentioned earlier. However, there was a notable decrease of 4% in onions grown in soil fertilized with the NPK chemical fertilizer, which contains predominantly potassium nitrate.

Table 4.5 Growth parameters of red onion grown for 3 months in non-fertilized (CTR), chemically (NPK), horse manure (HM), and AnchoisFert (AF) fertilizer.

Cation	*CTR [mg g⁻¹]*	*NPK [mg g⁻¹]*	*HM [mg g⁻¹]*	*AF [mg g⁻¹]*
NH_4^+	8.7 ± 0.5[d)]	10.3 ± 0.7[c)]	31 ± 2[a)]	20 ± 3[b)]
K^+	100 ± 4[a)]	96 ± 2[a)]	103 ± 3[a)]	102 ± 2[a)]
Mg^{2+}	4.6 ± 0.9[b)]	4.9 ± 0.8[b)]	7.9 ± 1[a)]	7.5 ± 2[a)]
Ca^{2+}	8.6 ± 1.2	10 ± 1.5[b)]	16 ± 2[a)]	15 ± 1[a)]
Anion	CTR [mg g⁻¹]	NPK [mg g⁻¹]	HM [mg g⁻¹]	AF [mg g⁻¹]
F^-	0.47 ± 0.02[a)]	0.34 ± 0.04[b)]	0.16 ± 0.03[c)]	0.29 ± 0.02[b)]
Cl^-	4 ± 0.5[b)]	16.4 ± 2[a)]	3.8 ± 0.9[b)]	19.4 ± 2[a)]
Br^-	n.a.	0.8 ± 0.3[a)]	n.a.	0.7 ± 0.4[a)]
NO_3^-	1.2 ± 0.5[a)]	0.7 ± 0.2[a)]	1.1 ± 0.6[a)]	0.7 ± 0.3[a)]
NO_2^-	5.9 ± 1[a)]	4.7 ± 0.8[a)]	3.9 ± 0.9[a)]	3.7 ± 0.7[a)]
SO_4^{2-}	1.9 ± 0.3[b)]	35 ± 1.2[a)]	2 ± 0.3[b)]	41 ± 2[a)]
Malate	2.8 ± 0.6[a,b)]	3.6 ± 0.7[a)]	2.1 ± 0.1[b)]	2.4 ± 0.2[b)]

[a)] Data expressed as mean of three replicates. Different letters indicate significant differences at $p < 0.05$.

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On the other hand, the amounts of magnesium (Mg^{2+}) and calcium (Ca^{2+}), which are highly beneficial for health, exhibited significant increases. For onions grown in soil fertilized with the new marine fertilizer (AnchoisFert), the amount of Mg^{2+} increased by 63% and Ca^{2+} increased by 74%. These increases were even greater in the presence of horse manure, with Mg^{2+} showing a 72% increase and Ca^{2+} showing an 86% increase.

In contrast, the amount of Mg^{2+} remained relatively unchanged (from 4.6 to 4.9 mg g^{-1}) for onions grown in the presence of the NPK chemical fertilizer. However, the amount of Ca^{2+} increased by 16%. These findings highlight the superior ability of the organic fertilizers, particularly AnchoisFert and horse manure, to enhance the uptake of magnesium and calcium by red onions compared to the NPK chemical fertilizer.

The analysis revealed that the amount of toxic fluoride and nitrate ions decreased in all fertilized bulbs compared to the control group. The decrease was particularly noticeable for fluoride in bulbs grown in soils fertilized with horse manure (HM), with levels decreasing from 0.47 mg g^{-1} to 0.16 mg g^{-1}. Similarly, bulbs grown in soils fertilized with AnchoisFert (AF) showed a decrease in fluoride levels from 0.47 mg g^{-1} to 0.29 mg g^{-1}.

The decrease in fluoride levels was more limited in the case of bulbs grown in soils fertilized with the NPK chemical fertilizer, with levels decreasing from 0.47 mg g^{-1} to 0.34 mg g^{-1}.

Regarding nitrate ions, the amount decreased from 1.2 mg g^{-1} to 0.7 mg g^{-1} for both AF and NPK fertilized bulbs. However, the decrease was negligible (from 1.2 mg g^{-1} to 1.1 mg g^{-1}) for bulbs fertilized with horse manure (HM), highlighting the high bioavailability of nitrogen present in the horse manure fertilizer.

These findings demonstrate that the use of organic fertilizers, particularly horse manure and AnchoisFert, effectively reduced the levels of toxic fluoride and nitrate ions in the bulbs, promoting the production of safer and healthier onions.

The analysis revealed that chloride levels increased in bulbs fertilized with the NPK chemical fertilizer, rising from 4 mg g^{-1} to 16.4 mg g^{-1}. However, bulbs fertilized with the new marine fertilizer AnchoisFert showed an even higher increase in chloride concentration, reaching 19.4 mg g^{-1}, which corresponds to a 4.85-fold increase.

Bromide was detected only in bulbs treated with the NPK chemical fertilizer (0.8 mg g^{-1}) and AnchoisFert (0.7 mg g^{-1}). This is likely due to the presence of bromide impurities in the NPK chemical fertilizer and the native bromide found in fish biowaste.

There were no significant differences in nitrate and phosphate levels between the control group and the different fertilization treatments. However, sulfate levels increased significantly in bulbs treated with both the NPK chemical fertilizer and AnchoisFert. In the NPK-treated bulbs, sulfate levels increased from 1.9 mg g^{-1} to 35 mg g^{-1} (an 18.4-fold increase), while in the

AnchoisFert-treated bulbs, sulfate levels increased from 1.9 mg g^{-1} to 41 mg g^{-1} (a 22-fold increase).

Regarding malate, there was a slight decrease in organically fertilized bulbs, while in the NPK-treated bulbs, there was an increase from 2.8 mg g^{-1} to 3.6 mg g^{-1} (a 28% increase).

These findings highlight the different effects of fertilizers on the levels of chloride, bromide, sulfate, and malate in the onion bulbs, indicating the importance of choosing appropriate fertilizers for specific nutrient requirements and avoiding the presence of impurities that can affect the composition of the plants.

Finally, the analysis of red onion bulbs (Table 4.6) showed that fertilization with different fertilizers had minimal effects on the protein, carbohydrate, and fiber content in the bulbs. However, there were significant increases in the total phenolic content (TPC) and total flavonoid content (TFC) in the fertilized bulbs compared to the control, with the organic fertilizers showing the greatest increases. In particular, onions fertilized with AnchoisFert exhibited a 66% increase in TPC, going from 14,040 μg TAE/g to 23,370 μg TAE/g. The TFC increased even more significantly by 89%, going from 2,601 μg QE/g to 4,914 μg QE/g. On the other hand, fertilization with horse manure (HM) resulted in a 35% increase in TPC and a 48% increase in TFC.

These findings indicate that organic fertilization, particularly with AnchoisFert, can significantly enhance the phenolic and flavonoid content in red onion bulbs, potentially leading to increased nutritional and health benefits.

Table 4.6 Total phenolic, flavonoid, protein and carbohydrate content, vitamin A, and fiber in red onion bulbs grown in pots for 3 months in non-fertilized (CTR), chemically (NPK), horse manure (HM), and AnchoisFert (AF) fertilized soils.

Parameter	*CTR*	*NPK*	*HM*	*AF*
Total phenols content [μg TAE/g]	14 040 ± 15[d)]	17 280 ± 33[c)]	18 930 ± 16[b)]	23 370 ± 27[a)]
Total flavonoid content [μg QE/g]	2601 ± 34[d)]	3305 ± 28[c)]	3866 ± 31[b)]	4914 ± 29[a)]
Vitamin A [μg g^{-1}]	0.17 ± 0.02[c)]	0.39 ± 0.03[a)]	0.25 ± 0.01[b)]	0.26 ± 0.03[b)]
Total protein [mg g^{-1}]	96 ± 2[b)]	105 ± 2[a)]	101 ± 4[a)]	97 ± 3[a)]

[a)] Data expressed as mean ± standard error. Different letters indicate significant differences at $p < 0.05$.

The fertilization with the NPK chemical fertilizer resulted in the lowest increase in total phenolic content (TPC) and total flavonoid content (TFC) compared to the control. The TPC increased by 23% and the TFC increased by 27% in the bulbs fertilized with NPK. However, it is worth noting that the NPK fertilizer led to the highest value of vitamin A (retinol) in the bulbs, reaching 0.39 mg

g^{-1}. Vitamin A is an essential nutrient with important roles in vision, immune function, and growth, among others. While the NPK fertilizer may have shown a relatively lower impact on the phenolic and flavonoid content, it provided a notable increase in vitamin A content in the red onion bulbs.

4.1.3 Experimental section

Elemental Composition, pH, and Electrical Conductivity:

Total and volatile solids, and pH were measured following established procedures [10]. The carbon and nitrogen content, expressed as the C/N ratio, was analyzed using a total organic carbon analyzer (TOC-LCSH) from Shimadzu [10].

The residual limonene in anchovy leftovers after fish oil extraction and solvent removal was analyzed using a previously reported procedure [11].

Electrical conductivity was determined using a conductivity meter (HI5522) from Hanna Instruments. A suspension of anchovy leftovers and water (at a ratio of 1:5) was mechanically shaken for 1 hour at 15 rpm to dissolve the water-soluble salts. The conductivity of the resulting solution was then measured using the conductivity meter. These analytical methods were employed to assess the elemental composition, pH, and electrical conductivity of the samples, providing important information about their chemical properties and characteristics.

Fertilization Experiment:

The fertilization experiments were conducted using pots filled with a sandy loam soil, which had specific proportions of clay, silt, and sand (11.85% clay, 23.21% silt, and 64.94% sand), according to the classification defined in the World Reference Base [12]. Each pot had a diameter of 30 cm and contained 9 kg of soil. The initial pH of the soil was 8.87, and it had an organic matter content of 1.81%.

AnchoisFert, the organic fertilizer derived from anchovy leftovers, was added to the pots at a concentration of 1.60 g based on its carbon content, which was reported to be 40%.

The experiment included three control groups: non-fertilized soil (CTR), soil fertilized with horse manure (Violmet Italy, Pisa), and soil fertilized with NPK (Agricoltura Italia, Taranto). These controls were used to compare the effects of AnchoisFert with commonly used organic and inorganic fertilizers. The experiments were performed in triplicates in a greenhouse, following a previously reported methodology [13].

Throughout the experiment, the pots were regularly watered to maintain a water content of 70% of field capacity. After 90 days of treatments, the soils from the different pots (three replicates) were air-dried and sieved to a particle size of less than 2 mm before conducting chemical analyses. Soil samples for biochemical determinations, such as microbial biomass and enzyme activities, were stored in a refrigerator at 4 °C for up to 24 hours before processing.

Soil Analysis:

To analyze the soil samples, various methods and instruments were used as described in the study:

1. Dry matter content: The dry matter content of both un-amended and amended soils was determined by heating the samples at 105 °C until the mass loss during 24 hours was lower than 0.5% of its weight.
2. Electrical conductivity: The electrical conductivity of soil samples was measured using a HI5522 conductivity meter (Hanna Instruments, Woonsocket, RI, USA). Samples were suspended in distilled water at a ratio of 1:5 (residue to water) and mechanically shaken at 15 rpm for 1 hour to dissolve soluble salts.
3. pH: The pH of the soil samples was measured in distilled water at a soil-to-water ratio of 1:2.5 using a HI 2210 glass electrode (Hanna Instruments, Woonsocket, RI, USA).
4. Organic carbon and total nitrogen: The content of organic carbon and total nitrogen in the soil samples was determined using conventional methods. Organic carbon was assessed using the dichromate oxidation method, while total nitrogen was measured using the Kjeldahl method.
5. Microbial biomass carbon (MBC): The microbial biomass carbon was determined in field moist samples (equivalent to

20 g dry weight) using Vance's extraction method [14]. The difference in organic carbon extracted from fumigated and unfumigated soil samples was used to estimate MBC. An extraction efficiency coefficient of 0.38 was applied to convert soluble carbon into biomass carbon [14].

6. Water-soluble phenols: Water-soluble phenols were extracted from the soil samples in triplicate using the method reported by Kaminsky and Muller [15]. The total water-soluble phenols, including monomeric and polyphenols, were determined using the Folin-Ciocalteu reagent. Tannic acid was used as a standard, and the concentration of water-soluble phenolic compounds was expressed as micrograms of tannic acid per gram of dry soil (μg TAE g^{-1} DS).
7. Fluorescein diacetate hydrolase (FDA): FDA hydrolysis, an indirect measure of total microbial activity, was determined according to the method of Adam and Duncan [16].
8. Dehydrogenase (DHA) activity: DHA activity, an indicator of microbial metabolic activity, was determined using the method of von Mersi and Schinner [17].
9. Cations and anions: Cations and anions in the soil samples were detected using ion chromatography with a Dionex ICS-1100 ion chromatograph (Thermo Fisher Scientific, Waltham, MA, USA). For anion analysis, dried material was stirred with an anion solution (Na_2CO_3/$NaHCO_3$ 3.5 mm) and the extract was filtered prior to chromatographic analysis. For

cation analysis, dry material was ashed at 550 °C, mineralized with concentrated HCl, and the resulting solution was filtered before ion chromatography analysis.

Plant Analysis:

To analyze the plant samples, the following methods and measurements were carried out:

1. Bulb diameter: The bulb diameter was measured using a caliper. This measurement provides an indication of the size of the onion bulb.
2. Leaf and root length: The length of both the leaves and roots of the red onion plants was measured using a meter. This measurement provides information about the growth and development of the plant's foliage and root system.
3. Harvest and separation: The plants were harvested when the bulbs reached maturity, characterized by neck softening and reduced solution uptake. The harvested plants were then separated into shoots, bulbs, and roots.
4. Fresh weight: The fresh weight of the entire plant and its individual parts (shoots, bulbs, and roots) was measured using a weighing scale. This measurement provides an indication of the biomass of the plant and its different components.
5. Drying: The separated plant parts (shoots, bulbs, and roots) were dried at 70 °C in an oven. Drying the samples removes moisture and allows for the determination of dry weights.

6. Dry weight: The dry weights of the plant parts were determined after the samples were dried. This measurement provides information on the biomass of the plant components after moisture has been removed.
7. Grinding: The dried plant materials were ground to pass through a 20-mesh sieve. Grinding the samples homogenizes the plant material and prepares it for further analysis.
8. Antioxidant compounds and activities: At the end of each growth cycle, the antioxidant compounds and antioxidant activities in the onion bulbs were measured. The specific methods for analyzing antioxidant compounds and activities were not mentioned in the given information, but these measurements are commonly conducted using various biochemical assays and spectrophotometric techniques to determine the levels of specific antioxidants and their activities.

These plant analysis methods and measurements were performed to assess the growth, biomass, and antioxidant characteristics of the red onion plants in response to the different fertilizer treatments.

Assessment of Phenolic Compounds in Bulb:

To assess the phenolic compounds in the red onion bulbs, the following methods were used:

1. Total phenol content: The Folin-Ciocalteu assay was adapted to measure the total phenol content in the red onion bulbs

[18]. The absorbance of each sample was recorded at 760 nm using a UV-1800 high-resolution spectrophotometer. A calibration curve was constructed using gallic acid as a standard, and the results were expressed as micrograms of tannic acid per gram of dried weight (DW). This assay provides an estimation of the total phenolic compounds present in the onion bulbs.

2. Total flavonoid content: The absorbance of the onion bulb extracts was measured at 430 nm to assess the total flavonoid content. A calibration curve was constructed using quercetin as a standard. The flavonoid content in the samples was calculated based on the calibration curve and expressed as micrograms of quercetin per gram of dried weight (DW). This measurement provides information about the total flavonoid content present in the onion bulbs.

These assays help quantify and evaluate the phenolic compounds, including both monomeric phenols and polyphenols, as well as flavonoids in the red onion bulbs. The results give insights into the levels of these bioactive compounds, which are known for their antioxidant and health-promoting properties.

Determination of Antioxidant Activities in Plants:

To assess the antioxidant activities in the plants, the following methods were employed:

1. DPPH radical-scavenging activity: The antioxidant activity against the DPPH radical was determined using a

spectrophotometric method [19]. The DPPH concentration in the cuvette was adjusted to yield absorbance values of approximately 1.0. Changes in absorbance of the violet solution were recorded at 517 nm after a 30-minute incubation at 37 °C. The inhibition of radical-scavenging activity (I%) was calculated using Equation (1):

$$I(\%) = [(A_0 - A_S)/A_0] \times 100 \quad \text{Eq(1)}$$

where A_0 represents the absorbance of the control and A_S represents the absorbance of the sample after incubation. This method helps evaluate the ability of the samples to scavenge the DPPH free radicals.

2. ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical cation decolorization assay: The antioxidant activity against the ABTS radical cation was determined by measuring the absorbance at 734 nm. The assay was conducted according to a published method [20]. The inhibition of radical-scavenging activity (I%) was calculated using the formula mentioned in the text, where A0 represents the absorbance of the control and AS represents the absorbance of the sample after incubation. The results are expressed as micromoles of Trolox equivalents per liter (μmol L−1 TE) using a Trolox calibration curve. This assay provides information on the ability of the samples to decolorize the ABTS radical cation.

1. Oxygen Radical Absorbance Capacity (ORAC-fluorescein) assay: The ORAC assay was performed according to a published method [21]. An aliquot of the extract was added to a fresh fluorescein solution and incubated for 15 minutes at 37 °C. Then, a sample of freshly prepared AAPH solution was added, and fluorescence was measured at regular intervals over a total analysis time of 90 minutes. The ORAC values, derived from a Trolox calibration curve, are expressed as equivalent Trolox micromoles per milligram of fresh weight. This assay helps measure the antioxidant capacity of the samples against peroxyl radicals.

These methods enable the evaluation of the antioxidant activities of the plant extracts by assessing their ability to scavenge free radicals and protect against oxidative stress. The results provide insights into the antioxidant potential of the plants and their potential health benefits.

Statistical Analysis:

The statistical analysis of the data was performed using analysis of variance (ANOVA) to assess the significance of the effects of fertilizers on each parameter measured. In addition, significant difference tests, such as t-tests, were conducted to compare the means between different treatments. The statistical software SPSS was utilized for these analyses [22]. The effects were deemed significant at a p-value of 0.05 or less, indicating a low probability of obtaining the observed results by chance. This rigorous statistical analysis helps determine the significance of the observed differences between treatments and provides confidence in the validity of the results.

4.2 Amino acids in new organic fertilizer AnchoisFert

The aim of this work is to identify the amino acids comprising the AnchoisFert proteins and assess their relative abundance. The analysis was carried out via a simple method based on their derivatization with ethyl chloroformate followed by gas chromatography-mass spectrometer (GC-MS) separation and identification of the resulting ethoxy carbonyl ethyl esters [23].

Involved in key metabolic processes vital to the health, growth, development and reproduction of organisms, amino acids are the building blocks of proteins and play an essential role in energy metabolism, neurotransmission, reproduction and immunity [24]. Not being synthetized by the human body, essential amino acids (EAAs) are entirely assumed through the diet by eating animal or vegetal foods rich in proteins, even though the nutritive value of vegetal proteins is lower due to lower or unbalanced EAAs content [25].

Anchovies, either fresh or processed (salted or canned), are a source of noble proteins widely consumed worldwide. For example, all the essential amino acids required in the human diet are present in fillets of Peruvian anchovy (*Engraulis ringens*) [26]. Similarly, researchers in Turkey recently identified nine EAAs (except from tryptophan) and nine non-essential amino acids in European anchovy (*Engraulis encrasicolus*) fished in the Aegean, Black and Marmara Seas, with lysine found in the highest amount in all groups [27]. Reduction of protein loss through valorization of by-products has long been identified as a key solution to reduce food loss and improve the sustainability of anchovy consumption [28].

4.2.2 Results and discussion

The AnchoisFert biomaterial consists of a grey powder of relatively small particles that can be freely handled even after drying (no static charge accumulation). Figure 4.3 shows the GC chromatogram profile of AnchoisFert following derivatization with ethyl chloroformate to convert its amino acids in readily separated ethoxy carbonyl ethyl esters. Table 4.7 shows the retention times and the ionic fragments for the 16 amino acids identified in AnchoisFert. Table 4.8 shows the relative abundance of the amino acids found in AnchoisFert.

Leucine (1.63 %), glycine (1.44 %), glutamic acid (1.21%) and alanine (0.88%) are the most abundant AAs in AnchoisFert.

Histidine (0.03 %), isoleucine (0.07 %), tyrosine (0.16%) and valine (0.14%) are the least plentiful. Lysine, that in fillets of fished in Turkey was the most abundant [27], has an intermediate concentration (0.32 %).

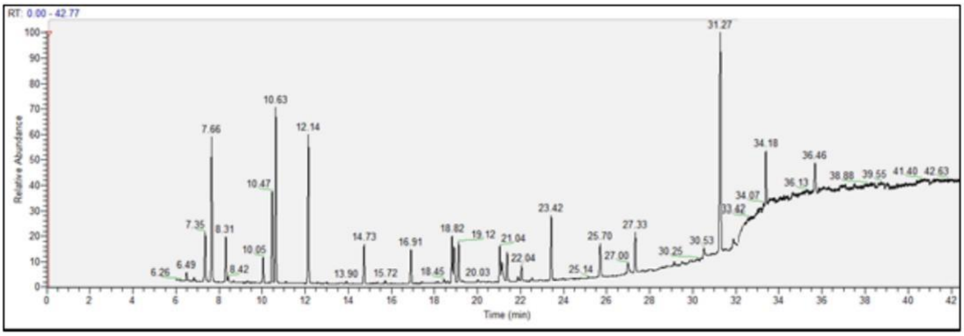

Figure 4.3 Chromatogram of AnchoisFert following derivatization of the AAs with ethyl chloroformate.

Glycine is a powerful anti-inflammatory immunonutrient [29], with a key structural (DNA, RNA and collagen synthesis), functional (heme, bile salts and creatin), and protective (glutathione and conjugation with drug molecules) role in human metabolism [30]. Leucine, an essential AA that provides a signal that amino acids are available stimulating muscle protein synthesis [31], is the second most abundant AA.

As put it by Jackson "only a small group of amino acids, alanine, aspartate and glutamic acid, are genuinely nonessential"[30]. The three NEEAs are particularly abundant in AnchoisFert. A component of dipeptide carnosine concentrated in muscle and brain tissue in humans, alanine is widely used as a strength-enhancing supplement [32]. In plants, alanine is accumulated as a generic stress response molecule involved in protecting plants from temperature extremes, hypoxia, drought, as well as chemical and biotic stresses [33].

Table 4.7 Retention time and ionic fragments for amino acids identified in AnchoisFert.

Amino acid	Retention time (min)	Ionic fragments (*m/z*)
Alanine	8.77	116/44
Valine	9.44	98/72/144/116
Isoleucine	10.78	74/158/130/102
Leucine	11.20	158/102/72/43
Glycine	11.93	102/74
Proline	13.46	142/98/70
Aspartic acid	20.25	188/116/42/74/56
Threonine	20.60	129/101/74
Methionine	22.53	175/61/129
Glutamic acid	22.72	84/41/56
Serine	22.93	132/129/60
Phenylalanine	24.95	176/192/91/102
Arginine	32.91	149/167/113/71
Lysine	35	156/128/45/226
Histidine	36.48	238/254/154
Tyrosine	38.57	107/192/264

Glutamic acid is the major neurotransmitter in humans, though it becomes toxic when present outside of protein in excess to dose healthy human can accommodate [34] (it is widely added as taste enhancer to many foods as sodium glutamate). Again, glutamate has a key signaling role in plants being involved in amino acid metabolism and was recently found to reshape the plant microbial community protecting plants against pathogens [35]. In addition, proline (0.87 %), aspartic acid (0.73 %), arginine (0.59%) and serine (0.31%) are also relatively abundant in AnchoisFert.

Table 4.8 Relative abundance of amino acids in AnchoisFert.

Amino acid	Abundance (wt %)
Alanine	0.88
Valine	0.14
Isoleucine	0.07
Leucine	1.63
Glycine	1.44
Proline	0.87
Aspartic acid	0.73
Threonine	0.23
Methionine	0.11
Glutamic acid	1.21
Serine	0.31
Phenylalanine	0.26
Arginine	0.59
Lysine	0.32
Histidine	0.03
Tyrosine	0.16
Total	8.97

Aspartic acid plays a critical role in protecting fish from bacterial infections by enhancing the concentration of nitrogen oxides that induce phagocytosis of microbial pathogens boosting fish immunity [36]. In higher plants, aspartate is the common precursor of the essential amino acids lysine, threonine, methionine and isoleucine, the lack of which dramatically reduces the nutritive value of all cereal and legume crops [37].

Dietary proline protects retinas from degeneration induced by the oxidative damage in the retinal pigment epithelium [38]. Accumulating in several plant species in response to environmental stress, proline plays a key role in plant recovery from stress [39].

Improving cardiovascular function and enhancing lean tissue mass, arginine is widely used as dietary supplement also to reduce obesity [40]. In plants, arginine, the AA with the highest N:C ratio amid the 21 proteinogenic amino acids, serves to store nitrogen as well as in defending plants against different stress agents [41].

Called "a metabolic hub" [42] and formed only in glial cells, serine links glial metabolism with synaptic activity and plasticity to such an extent that its lack contributes to many brain disorders. In plants, furthermore, serine has an important function in plant metabolism and development via both the photorespiratory glycolate pathway and the non-photorespiratory phosphorylated pathway [43].

Finally, lysine, the first limiting amino acid in nearly all developing countries where it is widely used today as dietary supplement to improve the nutritional status of populations [44], was found as mentioned above in intermediate levels (0.32 %). In higher plants, lysine enhances also the abiotic and biotic stress responses [45]. The aromatic amino acid phenylalanine (0.26 wt% in AnchoisFert) in plants exerts multiple biological functions and health-promoting properties, such as protection against abiotic and biotic stress as well as being required for protein biosynthesis and cell survival, acting as a precursor of numerous secondary metabolites [46].

4.2.3 Experimental section

Reagents and materials

All chemicals (reagent grade) were purchased from Sigma Aldrich (Milan, Italy) and used with further treatment processes.

Only chloroform was obtained from Fisher Chemical (Thermo Fisher Scientific, Rodano, MI, Italy). Ultrapure, de-ionized water was obtained with a Milli-Q water purification system (Merck-Millipore, Burlington, MA, USA).

Sample preparation

The AnchoisFert wet residue obtained after centrifugation of the AnchoisOil as described elsewhere [47], was washed on a Bruckner filter using a 583 micron nylon filter mesh filter first with pure EtOH (to remove residual limonene) and then with ultrapure water. The clean AnchoisFert obtained was thus dried in an oven at 110 °C for 2 h. A 5 mg sample of dried AnchoisFert was added to a vial followed by a 200 μL aliquot of concentrated (9 M) aqueous HCl.

Nitrogen gas was briefly insufflated with a glass pipette to remove oxygen from the mixture. The vial with the acidified sample was placed in a oven at 110 °C for 24 h, after which it was insufflated again with N_2, added with 300 μL ultrapure water and 300 μL of chloroform. The surnatant (50 μL) was transferred to another vial and added with 15 μL of aqueous $NaHCO_3$, 50 μL of ethanolpyridine (4 : 1, v : v), 10 μL of ethyl chloroformate, 50 μL of ethyl chloroformate with 1% internal standard (ethyl lactate) and 15 μL of aqueous $NaHCO_3$. After formation of two layers, a sample retrieved from the bottom layer was injected in the GC-MS spectrometer for the analysis.

GC-MS analysis

The GC-MS analysis was carried out using a Trace 1310 gas chromatograph with ISQ LT single quadrupole mass spectrometer CG-MS spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The GC was equipped with a Zebron ZB-WAX capillary column (bonded polyethylene glycol, 30 m×0.25 μm film thickness× 0.25 mm i.d.) supplied by Phenomenex (Torrance, CA, USA). A 1 μL sample was injected in split mode (1/100) using the Triplus RSH autosampler and liquid handling system (Thermo Fisher Scientific). High purity helium gas (99.999%) was used as carrier gas with a flow rate of 1.0 $mLmin^{-1}$. The oven temperature programming was as follows: the initial oven temperature held at 120°C for 2 min, then increased to 240°C at a rate of 4°$Cmin^{-1}$, and then to 260°C at a rate of 30°$Cmin^{-1}$ and hold for 10 min. The ion source and interface temperature were set at 280 °C and 265°C, respectively.

All samples were analyzed in selected ion monitoring (SIM) mode, in a mass range from 35 to 360 Da.

4.3 Conclusions

In this chapter, we have showcased the exceptional efficacy of "AnchoisFert," a solid residue obtained from milled anchovy leftovers after fish oil extraction using biobased limonene, as an organic fertilizer. We successfully demonstrated its ability to significantly enhance the growth of Tropea's red onion (*A. cepa*), using a lower quantity compared to horse manure (at approximately one-tenth of the amount). Our results clearly indicate that AnchoisFert outperforms commonly used organic (manure) and chemical (NPK) fertilizers in terms of promoting plant growth and development.

Indeed, farmers seeking economically viable organic fertilizers that can lead to higher yields of valuable horticultural crops will find AnchoisFert to be a highly advantageous option. This organic fertilizer provides essential soil and plant nutrients that effectively enhance the growth of red onions, resulting in a remarkable 65% increase in bulb weight and a substantial 133% increase in bulb diameter.

As a result, consumers can enjoy Tropea's red onions, which are already renowned for their health benefits due to elevated levels of flavonols and anthocyanins [48]. With AnchoisFert, these onions would exhibit a 66% increase in total phenolic content and a 75% increase in total flavonoid content, further enhancing their nutritional value.

The analysis via GC-MS of amino acids present in AnchoisFert unveils the presence of 16 amino acids, essential, quasi-essential and nonessential. Leucine, glycine, glutamic acid and alanine are the most abundant AAs. Proline, aspartic acid, arginine, serine, lysine and phenylalanine are also relatively plentiful.

Alongside the presence of abundant bioavailable organic carbon and valued minerals [49], the broad and significant role of virtually all these AAs in plant growth and metabolism further explains the exceptional fertilization properties of this new organic fertilizer.

AnchoisFert's composition, rich in proteins, organic carbon, flavonoids, magnesium, potassium, phosphate, and sulfate, makes it an excellent choice for promoting the healthy growth of various crops. It offers a viable alternative to both inorganic and organic fertilizers, allowing for sustainable and environmentally friendly agricultural practices.

The utilization of biobased limonene solvent derived from waste orange peel in a closed-loop production cycle marks a significant advancement in the sustainability of both fishing and agriculture. By shifting fish oil and fish-based fertilizer production from blue fish to blue fish waste [50], the process effectively closes the anchovy fishing material cycle. This approach not only minimizes waste but also creates a new class of organic fertilizers with exceptional performance by upgrading fish biowaste through a low-cost and environmentally friendly circular process.

The findings of the study indicate that AnchoisFert, the new organic fertilizer derived from anchovy biowaste, outperforms commonly used organic and inorganic fertilizers, even when applied in lower quantities. This highlights the effectiveness of AnchoisFert as a superior alternative for promoting plant growth and yield, specifically demonstrated in the case of Tropea's red onion.

Furthermore, the use of AnchoisFert as a replacement for manure does not contribute to an increase in the abundance or number of antibiotic resistance genes in the fertilized soil [51]. This is an important consideration for sustainable agricultural practices, as the emergence of antibiotic resistance poses a significant challenge to human and environmental health.

Additionally, the residual citrus limonene present in AnchoisFert acts as a natural fragrance and powerful antibacterial agent, limiting the formation of the unpleasant rotting fish odor that is typically associated with untreated fish biowaste. This makes AnchoisFert a more practical and user-friendly option for fertilizer application.

Overall, the use of AnchoisFert presents a promising solution for sustainable agriculture, offering superior performance, reduced environmental impact, and improved odor control compared to traditional fish waste-derived fertilizers.

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Chapter 5

Prospective life cycle assessment for the full valorization of anchovy fillet leftovers: the LimoFish process

5.1 The LimoFish process

Fish and seafood processing leftovers are widely recognized as a potential source of value-added biomolecules and biomaterials, such as bio-lubricants [1], biofuels [2][3], organic fertilizers [4] and bionano materials [5]. Fish processing increases the stability and quality of products, removing parts such as the viscera containing bacteria and enzymes that would otherwise quickly deteriorate proteins or lead to oxidation of the omega-3 polyunsaturated fatty acids (PUFAs) abundant in sea life lipids.

In 2011, more than 70 % of the total fish caught was subjected to processing before reaching the marketplace, with fish industry leftovers accounting up to 75 % (w/w) of the catch (depending on post-fishing and industrial processing processes) and on average 50 % in weight [6]. Since then, the share of processed fish has further increased due to economic and demographic development of numerous countries. In 2020, global fish production was 179 million tonnes [7], with half of it intended for human consumption, but the fish processing industry generates a significant amount of waste, producing 300–500 kg of waste per tonne of fish processed. After a slight reduction in 2020 likely due to the COVID-19 pandemic [8][9], fish catches increased again in subsequent years.

New sustainable management of seafood processing biowaste is a truly global problem [10]. Plentiful research efforts have been devoted to the valorization of this biowaste including for fish heads, tails, fins, skin and bones [11][5][12][13][14]. A few bioeconomy companies started the production of valued bio-products from fishery leftovers, especially fish oil and fish proteins for the pet-food industry [9], increasing the recovery of waste material and promoting circular economy strategies [15]. Rich in omega-3 lipids, indeed, the extraction of fish oil for the production of omega-3 food supplements significantly contributes to overfishing across the world [16].

Amid the new processes to recover valued bioproducts, the extraction of a whole fish oil named "AnchoisOil" (AnOil in the following) from Mediterranean anchovy (Engraulis encrasicolus) fillet leftovers using the bio-based solvent *d*-limonene was first reported in 2019 [17]. AnOil can replace fish oil (a cheap and convenient way to incorporate omega-3s into the diet) produced with conventional extraction and/or refining methods starting from the fatty tissues of oily fish such as salmon, mackerel, anchovies, and sardines.

D-limonene, a valued bio-based terpene, is commercially derived from orange and lemon peels prior to fruit squeezing for the production of citrus juice [18][19][20][21]. In contrast to petroleum-derived solvents, *d*-limonene is an edible substance with exquisite smell with multiple applications in the food and cosmetic industries.

Furthermore, the terpene has distinct antibacterial, antifungal, antioxidant and anticarcinogenic properties for which it is increasingly used in the nutraceutical and biopesticide industries [22][16]. Unlike low boiling solvents easily lost in the atmosphere such as widely employed *n*-hexane, *d*-limonene has a very high boiling point (176°C). The only, current limit of this extracting agent is its high upfront cost, mitigated by a unique stability that allows it to be reused several times with minimal quality losses [23].

The LimoFish process [22] is waste-free thanks to nearly complete recovery of the extraction solvent and to the use of the milled anchovy leftovers residues of the extraction as an exceptional organic fertilizer named "AnchoisFert" (AnFert in the following) [24]. Carried out under ultra-mild conditions (i.e., room temperature and atmospheric pressure) the process exemplifies a circular economy strategy applied to reduce the demand for natural resources (i.e., anchovies) and using anchovy fishery biowaste [25][26]. The process cuts significantly the cost of conventional energy-intensive fish oil extraction and refinement multistep processes by shifting the production of fish oil rich in omega-3 lipids from blue fish or dedicated cultivations [27] to blue fish leftovers.

5.2 The Life cycle assessment

A quantitative analysis of environmental impacts associated with the LimoFish has not yet been performed. Life cycle assessment (LCA) is a widely applied methodology to evaluate the environmental performance of a product, process, or service processes based on the assessment of direct and indirect impacts, and possible consequences [28]. Prospective (or looking-forward) LCA integrates forecasting methods in its approach to assess technology at an early-stage (i.e., lab scale, small scale production) to full scale-up implementation) [29][30][31][32][33][34][35].

Pereira da Silva et al. (2021) [33], for instance, integrated LCA evaluation in the development stage of a process aimed at extracting starch from mango kernel. On a laboratory scale, the LCA methodology allows to identify the potential environmental hotspots and provide suggestions useful for supporting future design. On the other hand, its adoption on industrial scale can be used to assess impacts under operating conditions close to the real process (e.g., energy use, emissions, and waste disposal). The application of the LCA at both scales can identify potential trade-offs and foster collaboration among stakeholders.

In this study, we apply LCA to evaluate the potential environmental burdens associated to the AnFert and AnOil production via the LimoFish process in order to estimate the environmental impacts of the secondary production (i.e., not from dedicated systems). LCA was first applied to laboratory data. Then a scale-up of the novel process modelled according to well-established methods [36][37] was evaluated using the LCA approach for modelling the production of chemicals for the overall evaluation of the process at industrial level.

According to the international standards ISO 14040-14044 [28][38] LCA is a strategic technique to identify and quantify the potential environmental impacts associated with a product, process or system throughout its life cycle. The common LCA framework (goal and scope definition, life cycle inventory - LCI, and life cycle impact assessment - LCIA), applies environmental mechanisms and characterization models to relate the LCI results to selected category indicators for a quantitative evaluation of environmental impacts. A fourth phase, i.e., interpretation, transversal to the previous three, ensures consistency between the aims of the study and its execution to finally draw recommendations. In the following paragraphs, the four phases are described with reference to the LimoFish process.

4.2.1 LCA definitions and system boundaries

Figure 5.1 depicts the main stages of the LimoFish at the laboratory scale (LabS) and after scale-up (SUp).

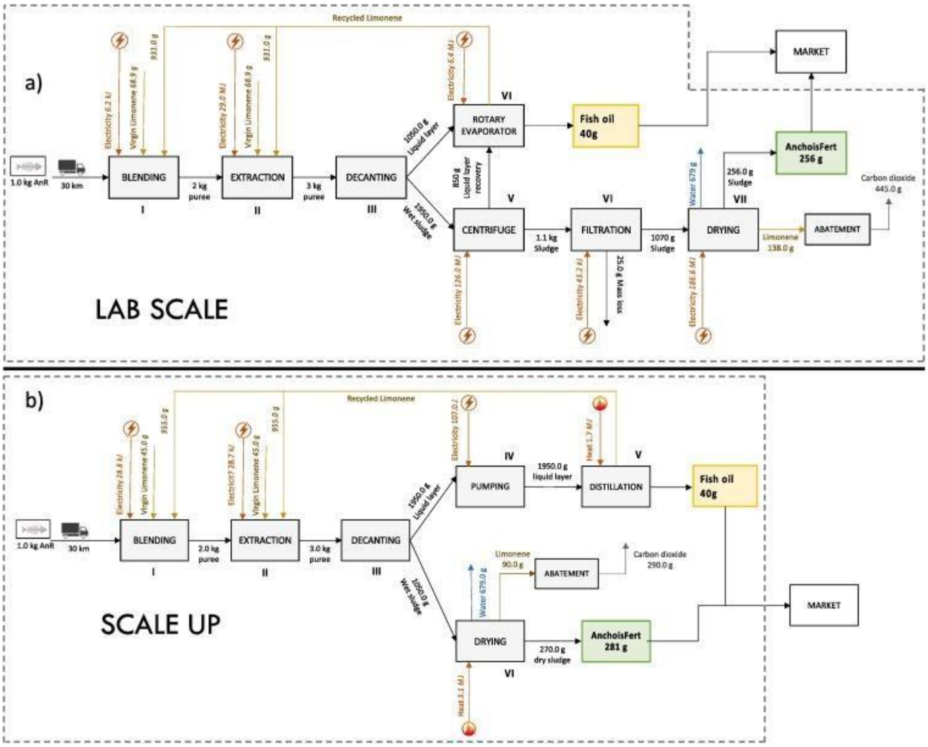

Figure 5.1 System boundaries for each model. (a) LabS and (b) SUp.

Both operating scales are modelled by LCA in this study. The functional unit (FU) of the study was set at 1 kg of anchovy leftovers inflow subjected to the LimoFish. The choice of using 1 kg of inflow material instead of 1 kg of product is made in order to focus the problem on the waste valorization, according to the main literature on waste management [39][40][41]. The system boundaries (Figure 5.1) were defined following a "cradle-to-gate" approach, including therefore production and supply of raw materials and chemicals, energy requirements, and waste management occurring along the process. The infrastructure was excluded from the consideration, since its contribution is negligible for the case study.

According to ecoinvent data [42], a reasonable estimation of a chemical working plant, assuming an average lifetime of 30 years [42] and an annual production of 810kt [42] of a generic chemical, is 1.35E-03 kgCO2eq/kg of produced material. This value is accordingly considered not significant. The total impacts associated to the FU were calculated and a contribution analysis is provided with the aim to determine the most relevant steps and their influence on the environmental sustainability of the innovative fish oil and organic fertilizer production. A sensitivity analysis was thus performed with the aim to test the robustness of the model as well as to evaluate the influence of the assumptions on the final outcomes.

5.2.1 Life cycle inventory (LCI)

The system under scrutiny consists of a sequence of processes aimed at extracting the valued AnOil obtaining AnFert as co-product. Primary data from laboratory experiments were collected to fill mass balances in the case of LabS scenario. Dedicated checklists were adopted. On the other hand, for the SUp modelling we had to face one of the most challenging steps of the prospective LCA, that is the unavailability of life cycle inventory data [43]. In facts, in the field of chemical engineering, SUp is a decisive and integral part and many authors dedicated time and resources to this area, since the upscaling normally consists in several steps before the actual plant is built [37].

According to Piccinno et al. (2016) [37], LabS steps must be converted into larger scale reactors, apparatus and main equipment. It is specified that overall goal of the developed SUp framework is to allow a simulation of the process, following an LCA perspective and referring to logically and systematically compiled data. In this view, some simplifications had to be made to adapt the described framework. For this reason, the inventory was elaborated referring to the methodologies described by Maranghi et al. (2020) [36] and Piccinno et al. (2016) [37] and compiled using secondary (background) data based on the relevant literature in the field or from the ecoinvent 3.7 database [42].

The main adaptation from the articles of Maranghi and Piccinno is related to the choice of reactor/equipment, in replacement of each laboratory phase (see Paragraph 4.2.2). Materials and energy flows involved in each step of the system were reported in Table 5.1, while proxy processes drawn from ecoinvent are listed in Table 5.2. The detailed description of the main assumptions and of the product system is as follows:

Table 5.1 Data inventory of the LimoFish.

LabS		**SUp**	
Inbound transportation	30 km	Inbound transportation	30 km
Blending	6.2 kJ_e* 68.9 g of *d*-limonene	Blending	28.8 kJ_e 45 g of *d*-limonene
Extraction	29.0 MJ_e 68.9 g of *d*-limonene	Extraction	28.7 kJ_e 45 g of *d*-limonene
Decanting	/	Decanting	/
Centrifuge	126.0 MJ_e	Pumping	107.1 J_e
Rotary evaporator	6.4 MJ_e	Distillation	1.7 MJ_t*
Filtration	43.2 kJ_e	/	/
Drying	186.6 MJ_e	Drying	3.1 MJ_t

*Letters e and t refer to electric and thermal energy respectively.

(i) Since the end-of-life management applied to the amount of AnLeft used as primary source for the AnOil is currently not known, it is not possible to provide estimations about the benefits and/or burdens that its management would imply. For this reason, it was decided to apply the conservative "zero burden" criterion, and no environmental impacts were attributed to its production. However, since its supply is necessary to allow the functioning of the system, the contribution due to transportation of the

AnLeft is included in system boundaries, by assuming 30 km distance covered by truck.

Table 5.2 Ecoinvent reference processes.

ITEM	Unit	Ecoinvent Process
Water	kg	*Tap water {CH}| market for | APOS, U*
Anaerobic digestion emissions (LabS)	m^3	*Biogas {CH}| anaerobic digestion of manure | APOS, U* *iii
Anaerobic digestion emissions (SUp)	m^3	*Biogas {CH}| anaerobic digestion of manure | APOS, U* *iii
Cogeneration emissions	MJ	*Electricity, high voltage {IT}| heat and power co-generation, biogas, gas engine | APOS, U* *iii
d-Limonene (emission to air)	kg	*d-Limonene to air*
Electricity consumption	MJ	*Electricity, medium voltage, at grid/IT U*
Electricity production	MJ	*Electricity mix/IT U*
Exhausted filter management	kg	*Waste paper, unsorted {CH}| market for | APOS, U*
Fertilizer NPK	kg	*NPK (15–15–15) fertiliser {RER}| market for NPK (15–15–15) fertiliser | APOS, U*
Heat	MJ	*Heat, central or small-scale, natural gas {RER}| market group for | APOS, U*
Transportation (with refrigeration)	tkm	*Transport, freight, lorry with refrigeration machine, 3.5–7.5 ton, EURO5, R134a refrigerant, freezing {GLO}| transport, freight, lorry with refrigeration machine, 3.5–7.5 ton,*

ITEM	Unit	Ecoinvent Process
		EURO5, R134a refrigerant, freezing | APOS, U

(ii) The environmental impacts of the electricity consumption were calculated according to the Italian electrical energy mix in 2020 [44], consistent with the geographical boundaries of the system investigated.

(iii) In LabS case the biogas process of ecoinvent was taken as reference only to estimate the air emissions. Electricity consumption and the production of the waste inflow were excluded, since already included in the model as primary data. The same was done for the SUp scenario. A similar approach was applied to the cogeneration phase, where the impacts associated to the biogas inflow were accordingly substituted from primary data.

(iv) To calculate the potential environmental impacts of the extracting agent (*d*-limonene), the inventory data were adapted from Santiago et al., (2020) [20] considering two production techniques: namely, hydrodistillation and cold pressing from waste orange peel. Hydrodistillation affords a higher production yield (4.5 $kg_{d\text{-limonene}}$/100 kg_{waste}) and lower water consumption (1 g_{water}/100 kg_{waste}). Cold pressing affords a production yield of 3.3 $kg_{d\text{-}limonene}$/100 kg_{waste} and a water consumption of 5.4 kg_{water}/100 kg_{waste}. Cold pressing, however, requires significantly lower energy (101.6 MJe and 1.0 MJt, respectively) than hydrodistillation (29.3 MJ_e and 1607.9 MJ_t). In light of its high yield, we selected hydrodistillation as the production route of choice. The inventory was modeled accordingly using the LCA Software of SimaPro 9.2 [45], considering the Italian energy mix for the electricity [44] and natural gas as primary source for heat (Table 5.3 and Table 5.4).

Table 5.3 Limonene production inventory (hydrodistillation).

INPUT	***Category***	***Amount***	***Unit***
Crusher	Electricity	30,00	kWh
Conveyor	Electricity	6,50	kWh
Bucket Conveyor	Electricity	0,08	kWh
Pump Extraction	Electricity	0,07	kWh
Pump Purification	Electricity	0,03	kWh
Decantation Unit	Electricity	0,00	kWh
Biogas AP	Electricity	0,00	kWh
Total	***Electricity***	**36,69**	***kWh***
Extraction Unit	Heat	717,40	kWh
Condenser Purification	Heat	716,10	kWh
Condenser Purification 2	Heat	281,20	kWh
Reboiler	Heat	294,10	kWh
Condenser	Heat	0,03	kWh
CSTR	Heat	1,00	kWh
Total	***Heat***	***2009,83***	***kWh***
Tap water	***Water***	***0,00***	***kg***

OUTPUT	***Category***	***Amount***	***Unit***
Biogas- citrus waste	Material	4,30	Nm^3
d-Limonene	Material	4,50	kg
Digestate - Citrus waste	Material	68,60	kg
Wastewater - anaerobic digestion	Material	**15,30**	kg

Economic allocation	**Amount**	**€/kg (€/Nm^3)**	**€**
Limonene	4,5	37,3	167,85
Biogas	4,3	0,01	0,043
Digestate	68,6	23	1577,8

Table 5.4 Limonene production inventory (cold pressing).

INPUT	***Category***	***Amount***	***Unit***
Crusher	Electricity	30,00	kWh
Conveyor	Electricity	6,50	kWh
Bucket Conveyor	Electricity	0,08	kWh
Centrifugation Purification	Electricity	3,70	kWh
Pump Purification	Electricity	0,01	kWh
Decantation Unit	Electricity	1,90	kWh
Biogas AP	Electricity	0,00	kWh
Extraction Unit	Electricity	59,40	kWh
Pump Extraction	Electricity	0,00	kWh
Total	***Electricity***	**101,59**	***kWh***
Condenser	Heat	0,03	kWh
CSTR	Heat	1,00	kWh
Total	***Heat***	**1,03**	***kWh***
Tap water	***Water***	**17,70**	***kg***
OUTPUT	***Category***	***Amount***	***Unit***
Biogas- citrus waste	Material	4,30	Nm^3

d-Limonene	Material	3,30	kg
Digestate - Citrus waste	Material	68,60	kg
Wastewater - anaerobic digestion	Material	**50,60**	kg

Economic allocation	**Amount**	**€/kg (€/Nm³)**	**€**	**%**
Limonene	3,3	37,3	123,09	7,24%
Biogas	4,3	0,01	0,043	0,00%
Digestate	68,6	23	1577,8	92,76%

(v) Santiago et al., (2020) [20] simulated the co-production of *d*-limonene from citrus waste, together with biogas and digestate. Therefore, in our simulation an economic allocation of 9.6 % was applied to the total impact to simulate the environmental burdens due to *d*-limonene production. The influence of biosolvent production technology choice on the final results was consistently tested in the sensitivity analysis. Being derived from biowaste, the aforementioned zero burden criterion was applied to raw source of *d*-limonene, waste orange peel [20].

(vi) The electricity flows were not directly measured during the AnOil extraction. Hence, the electricity consumption (EC) of each step of the LabS process was calculated in accordance with Equation (1):

$$EC\ (MJ) = P\ (kW) \cdot t(h) \cdot 0.3 \cdot 3.6\ (\frac{MJ}{kWh}) \quad \text{(Eq.1)}$$

where P is the equipment power consumption (in kW), when used at its maximum power, t is the working time (in h) and 3.6 is the converting factor from kWh to MJ. The correction factor 0.3 is arbitrarily fixed, assuming that each specific equipment operates at the 30 % of its potential due as a consequence of its oversize in relationship to real dimensions of the experiment. The impact of this assumption on the final results is discussed in the paragraph 4.4. For the SU simulation, electricity and heat consumptions were estimated following the approach of Piccinno et al. (2016) [37]. For more details see Table 5.5 (LabS electricity consumption) and Tables 5.5, 5.6, 5.7, 5.8, 5.9, 5.10, 5.11 (up-scaling). A detailed description of the whole product-system is reported below. The relevance to LabS and/or SUp is in accordance with Piccinno and co-workers.

Table 5.5 LabS electricity consumption.

Process	Power (W)	Time of use	Time of use (h)	Energy (Wh)	Energy (MJ)	Energy (MJ) 30%	Energy (MJ) 50%
Blending	**230**	**45 s**	**0,01**	**2,88**	**0,01**	**0,003**	**0,01**
Extraction	**640**	**21h, 700 rpm, 25°**	**21,00**	**13440,00**	**48,38**	**14,515**	**24,19**
Decanting	**/**	**/**	**/**	**/**	**/**	**/**	**/**
Rotary Evaporator	**5921**	**30 mins**	**0,50**	**2960,50**	**10,66**	**3,197**	**5,33**
Water bath 1000 W	*1000*	/	/	/	/	/	/
Rotating arm 45 W	*45*	/	/	/	/	/	/
Pump 220-240 W	*230*	/	/	/	/	/	/
Refrigerant 2530 W (230 V, 11A)	*2530*	/	/	/	/	/	/
Refrigerant computer 2116 W (230V, 9.2 A)	*2116*	/	/	/	/	/	/
Centrifuge	**350**	**10 mins, 10000 rpm**	**0,17**	**58,33**	**0,21**	**0,063**	**0,11**
Filtration	**240**	**5 mins**	**0,08**	**20,00**	**0,07**	**0,022**	**0,04**
Oven (drying)	**1200**	**72**	**72,00**	**86400,00**	**311,04**	**93,312**	**155,52**
Anaerobic digestion	**640**	**Oven (1 month, 35°)**	**720,00**	**460800,00**	**1658,88**	**497,664**	**829,44**
Oven 400 W	*400*	/	/	/	/	/	/
Stirrer 240 W	*240*	/	/	/	/	/	/

Table 5.6 SUp blending.

Input	**Amount**	**Unit**
Frozen Anchovy Leftovers puree	1000,00	kg
Recycled D-Limonene	1910,00	kg
D-Limonene	90,00	kg
Electricity	2,87	MJ
Output	**Amount**	**Unit**
Frozen Anchovy Leftovers puree + D-Limonene	3000,00	kg

% D-Limonene Recycled	0,96	/

Electricity for Stirring		**2,87**	**MJ**
Reactor size	/	5,00	m3
Power number of impeller	Np*	0,79	-
total mass (D-Limonene + Anchovy puree)	/	2000,00	kg
D-Limonene Mass	/	1000,00	kg
D-Limonene Density	/	841,00	kg/m³
total mass (D-Limonene + Anchovy puree)	/	2000,00	kg
D-Limonene Volume (m/d)	/	1,19	m³
Density of the reaction mixture (total mass/V Lim)	ρ mix	1682,00	kg/m³
Reaction time (1h=3600s) - 12h	t	32400,00	s
Efficiency	η stir*	0,90	-

Table 5.7 SUp extraction.

Input	**Amount**	**Unit**
Frozen Anchovy Leftovers puree	1000,00	kg
Recycled D-Limonene	1910,00	kg
D-Limonene	90,00	kg
Electricity	2,87	MJ
Output	**Amount**	**Unit**
Frozen Anchovy Leftovers puree + D-Limonene	3000,00	kg

% D-Limonene Recycled	0,96	/

Electricity for Stirring		**2,87**	**MJ**
Reactor size	/	5,00	m3
Power number of impeller	Np*	0,79	-
total mass (D-Limonene + Anchovy puree)	/	2000,00	kg

D-Limonene Mass	/	1000,00	kg
D-Limonene Density	/	841,00	kg/m³
total mass (D-Limonene + Anchovy puree)	/	2000,00	kg
D-Limonene Volume (m/d)	/	1,19	m³
Density of the reaction mixture (total mass/V Lim)	ρ mix	1682,00	kg/m³
Reaction time (1h=3600s) - 12h	t	32400,00	s
Efficiency	η stir*	0,90	-

Table 5.8 SUp decanting.

Input	***Amount***	***Unit***
Frozen Anchovy Leftovers puree + D-Limonene	3000,00	kg
Output	**Amount**	**Unit**
Extract Solution	1950,00	kg
Sludge	1050,00	kg

Table 5.9 SUp pumping.

Input	**Amount**	**Unit**
Extract Solution	1950,00	kg
Electricity	0,11	MJ
Output	**Amount**	**Unit**
Pumped-Extract Solution	1950,00	kg

Electricity for pumping		**0,11**	**MJ**
Transferred mass (ton of pumped material)	m	1950,00	kg
Transferred mass (kg of pumped material)	m	1950,00	kg
Gravitational acceleration	g	9,81	m/s
Height difference	Δh*	4,20	m
Efficiency	η pump*	0,75	-

Table 5.10 SUp distillation.

Input	**Amount**	**Unit**
Pumped-Extract Solution	1950,00	kg
Heat	1654,95	MJ
Output	**Amount**	**Unit**
D-Limonene Recycled	1910,00	kg
Fish oil	40,00	kg

Heat of distillation		**1654,95**	**MJ**
Reactor size		5000,00	L
Molar heat capacity of the solvent (D-Limonene) g=mol/PM	Cp mol	249,40	J/(mol*K)
PM D-Limonene		0,14	kg/mol
PM D-Limonene		0,14	kg/mol
Specific heat capacity of the solvent (D-Limonene)	Cp_solv	1830,73	J/(kg*K)
Mass of the reaction mixture (mmix)	m_mix	1950,00	kg
	m_mix	1950,00	kg
D-Limonene (solvent) boiling Temperature 177°C = 450.15 K	T_boil	450,15	K
T_0 usually ambient temperature 25°C = 298.15 K	T_0	298,15	K
	ΔvapH_1	38,65	kJ/mol
	ΔvapH_2	38650,00	J/mol
enthalpy of vaporization (DHvap [J/kg]) (D-Limonene)	Δh_vap	283711,37	J/t
the mass of the liquid (mdist) that needs to be vaporized	m_dist	1910,00	kg
	m_dist	1910,00	kg
relative volatility of the solvents (>1);	α	54,51	-
target purity of distillate (molar fraction)	X_LD	1,00	-
molar fraction of target compound in feed.	X_LF	0,50	-
	1/a-1	0,02	-
	Xld/Xlf	2,00	-
	A*(1-Xld)/(1-Xlf)	0,00	-
reflux ratio	R_min	0,04	
heating element's efficiency	η_heat*	0,77	-

Table 5.11 SUp drying.

Input	**Amount**	**Unit**
Heat	3085,98	MJ
Sludge - Decanting	1050,00	kg
Output	**Amount**	**Unit**
Dry Sludge	0,18	kg
Water (to air)	959,82	kg
D-Limonene (to air)	90,00	kg

Heat of drying	**Q_dry**	**3085,98**	**MJ**
	Q_dry	3085977943,97	J
specifc heat capacity water	C_(p,liq)	4184,00	J/(kg*K)
mass of water (mass of the solvent to be evaporated)	m_liq	959,82	kg
	m_liq	959,82	Kg
T_boil solvent (water) = 100°C	T_boil	373,15	K
T_0 = 25°C	T_0	298,15	K
	ΔH_vap	40,65	kJ/mol
molecular mass water	ΔH_vap	40650,00	J/mol
	PM water	18,00	g/mol
	PM water	0,02	kg/mol
enthalpy of vaporization (DHvap [J/kg])	ΔH_vap	2258333,33	J/kg
mass of vapor	m_vap	959,82	kg
dryer Efficiency	η_dry	0,80	-

0) Raw material supply and inbound transportation.

Having a biorefinery located within a few km is important for reducing transportation costs and emissions, increasing efficiency, and creating a circular economy by utilizing waste streams. This evidence is particularly crucial for feedstocks with high water content, where transportation can significantly impact both the economy and the environment, especially when considering carbon footprint. Literature suggests that optimal distances for transportation range between 7.5 and 100 km [46]. The AnLeft is therefore assumed to be delivered by a freight truck equipped with a refrigerator from a supplier located 30 km away from the LimoFish production plant. The impacts associated to the transportation (tkm) were gathered from the ecoinvent database [42]. The oil:solid:water composition ratio to extract 1 kg of AnLeft was set at 0.04:0.28:0.98, according to the LabS conditions.

I) Blending (LabS and SUp).

The process is required to homogenize the AnLeft. In the SUp, the blending step is performed with a rotor–stator homogenizer of 5000 L. The parameters related to dimensions and technology were reported in Piccinno et al. (2016) [37]. The stirring time was fixed at 9 h [22]. The electricity input to allow a proper mixing of 1 kg of AnLeft is 6.2 kJ_e/FU in the LabS while the SUp value of 28.8 kJe/FU is calculated according to Equation (2), in which the type of the impeller (N_p), the diameter of the impeller (d), the stirring rotational velocity (N), the density of the reaction mixture (ρ_{mix}), the reaction time (t) and an efficiency value (η_{stir}) were considered [37]. The virgin (68.8 g/FU on LabS and 45 g/FU on SUp) and recycled *d*-limonene (1.9 kg/FU on LabS and 1.2 kg/FU on SUp) were entered at 50 % rate in the blending step and at 50 % rate in the extraction step. For both the LabS and SUp the output of the blending process was 2 kg/FU of anchovy leftovers puree at low grade.

Equation (2): SUp blending.

$$E_{stir}[J] = \frac{N_p * \rho_{mix}(kg/m^2) * N^3 (1/s) * d^5 (m) * t(s)}{\eta_{stir}} \quad \text{(Eq.2)}$$

II) Extraction (LabS and SUp).

This step was applied to extract the fish oil fraction from the AnLeft puree (3 kg) and separate it from the other components (e.g., *d*-limonene, water, impurities). Also in this case the process was performed in a rotor–stator homogenizer of 5000 L, assuming the same conditions of the blending step but with a reaction time of 12 h [22]. The electricity consumption of the process is 29.0 MJ_e/FU for the LabS and 28.8 kJ_e/FU for the SUp (Equation (2)). For both LabS and SUp steps of the process, the mix of puree and *d*-limonene output was sent to decanting. For the SUp process, the extract (*d*-limonene + AnOil) was then directed to centrifuge and the wet sludge to the drying phase.

III) Decanting (LabS and SUp). The aim of this process is to separate the two different fractions present in the 3 kg of puree deriving from the extraction step. The decantation step does not require energy or external chemical inputs. The liquid fraction (1.05 kg/FU for LabS and 1.95 kg/ FU for SUp), contains the fish oil dissolved in *d*-limonene. The wet sludge (1.95 kg/FU for LabS and 1.05 kg/FU for SUp), contains water and *d*-limonene. The *d*-limonene:water ratio in the drying stage was calculated at 1:4.9 in the LabS and 1:7.5 in the SUp, due the higher efficiency of the recovery at the industrial scale.

IV) Rotary evaporation (LabS) and Pumping (SUp). The liquid fraction separated by decantation and centrifugation constitutes the input to rotary evaporation. This stage enables to recover fish oil (40 g/ FU) and *d*-limonene. The purity of the recovered *d*-limonene was monitored by using an off-line gas chromatograph (Agilent 6890 N) equipped with a wide-bore capillary column (CP-WAX 52CB, 60 m, i.d. 0.53 mm) and a flame ionization detector (FID). A purity higher than 95 % was always detected, aligning our findings with similar results in the literature [47]. In addition, in the laboratory tests the recovered *d*-limonene via evaporation under reduced pressure was reused up to 4 times without a significant loss in its extraction ability. Concerning the SUp, a pumping stage is needed to convey 1.95 kg of liquid fraction from the decanting step to the distillation step. The electricity consumption, calculated according to Equation 2 [37], resulted in 10.7 J_e/FU. The expression considers the transferred mass (m), the gravity acceleration (g), an assumed height difference (Δh) and the pumping efficiency (ηpump):

Equation (3): SUp pumping.

$$E_{pump}[J] = \frac{m(kg)*g(m/s)*\Delta h(m)}{\eta_{pump}} \quad \text{(Eq. 3)}$$

In agreement with what was observed when extracting rice bran oil from rice bran [47] when the amount of oxidized *d*-limonene remained less than 1 % even after several extraction and biosolvent recovery cycles, in the LimoFish process, the *d*-limonene recovered via evaporation under reduced pressure (40 mbar) at relatively high temperature (90°C) remained pure at reusable at length, as confirmed by GC–MS analysis.

V) Centrifuge (LabS) and Distillation (SUp).

The goal of this step is to separate the liquid and the solid fractions of the wet sludge deriving from the decanting step. On a lab-scale, centrifuge requires an electricity input of 126 J_e/FU and affords a liquid layer (850.0 g, sent to the rotary evaporator) and a sludge that in its turn was directed to the filtration step. In the SUp process, rotary evaporation is replaced by a distillation reactor that is one of the most energy demanding steps in the overall fish oil and organic fertilizer production route (with 1.7 MJ_t/FU energy demand). The energy consumption was estimated by Equation (4) and (5) [37]. The parameters considered are: the heat capacity (C_p), the mass of reaction mixture (mmix), the *d*-limonene boiling temperature (T_{boil}), the ambient temperature (T_0), the enthalpy of vaporization of *d*-limonene (Δh_{vap}), the mass of the liquid that needs to be evaporated (m_{dist}), the reflux ration (R_{min}, calculated by Equation (5)) and the heating element's efficiency (η_{heat}). On the other hand, Rmin was calculated by knowing the relative volatility of the solvents (α), the target purity of distillate (XLD) and the molar fraction of the target compound in feed (XLF) [37]. Distillation enables to obtain the final 40.0 g/FU of AnOil and to recycle 1.2 kg/FU of the solvent.

Equation (4): SUp distillation.

$$Q_{dist}\,[J] = \frac{C_p(J/kg*K)*m_{mix}(kg)*(T_{boil}(K) - T_0(K)) + \Delta h_{vap}(J/T)*m_{dist}(kg)*(1.2*R_{min} + 1)}{\eta_{heat} - 0.1} \quad \text{(Eq. 4)}$$

Equation (5): SUp, calculation of R_{min} for Q_{dist} estimation.

$$R_{min} = \frac{1}{\alpha - 1}*[\frac{X_{LD}}{X_{LF}} - \frac{\alpha*(1 - X_{LD})}{1 - X_{LF}} \quad \text{(Eq. 5)}$$

On large scale, the milled anchovy leftover solid residue after extraction readily precipitates on the vessel of the extraction vessel when stirring is stopped. No centrifugation and/or filtration is required to separate the liquid phase comprised of *d*-limonene and the extracted lipids and vitamins from the solid phase. The liquid phase is then simply recovered by decantation.

VI) Filtration (LabS).

The sludge obtained from the centrifuge on LabS is filtered, with an energy consumption of 43.2 J_e/FU. The waste stream of this step, comprised of the exhausted filter and vapor mass loss (30.0 g), was assumed to be managed as "hazardous waste", with emissions modelling from the ecoinvent database [42].

VII) Drying (LabS).

This step is required to evaporate the water fraction and the residual *d*-limonene (not recycled) in the sludge from the pumping step. The heat consumption of the oven was estimated to be 3.1 MJ_t/FU. The amount of evaporated *d*-limonene, estimated to be 90.0 g/FU trough the mass balance, was burned and assumed to be totally converted in CO_2 (stoichiometric approach) emitted in the atmosphere.

VIII) Drying (SUp).

As in the previous stage, it is required to remove water and *d*-limonene traces from the sludge. In the SUp process, the drying phase can remove the majority of *d*-limonene. The amount of evaporated biosolvent, estimated to be 137.8 g/FU, was also burned converted in CO_2. The electricity consumption of the oven was estimated with Equation (6) [37] and evaluated equal to 186 MJ_e/FU. The parameters considered in the equation were the specific heat capacity of water ($C_{p,liq}$), the mass of water to be evaporated (m_{liq}), the boiling temperature of water (T_{boil}), the ambient temperature (T_0) and the enthalpy of vaporization (Δh_{vap}). After this step, the dried AnFert can be directly employed as an organic fertilizer without any further treatment replacing a commercial inorganic fertilizer (NPK, 20:10:10) [24].

Equation (6): SUp drying.

$$Q_{dry}(J) = \frac{C_{p,liq}(J/kg*K)*m_{liq}(kg)*(T_{boil}(K) - T_0(K)) + \Delta h_{vap}(J/kg)*m_{vap}(kg)}{\eta_{dry}} \quad \text{(Eq. 6)}$$

5.2.2 Life cycle impact assessment (LCIA)

The LCIA phase includes quantitative assessment of the potential environmental impacts resulting from the system under scrutiny. To this aim, environmental mechanisms and characterization models were applied to relate the LCI results to selected categories. The IMPACT World + method [48], one of the most comprehensive and recent LCA methods for environmental impact evaluations [49][50][51], was selected for the study. The

LCIA method is employed to determine the impacts associated to the LimoFish process (LabS and SUp).

5.2.3 Allocation criteria

Due to the absence of economic information related to AnOil fish oil and AnFert organic fertilizer, new products obtained through the LimoFish process, no allocation criteria can be applied to the two products obtained. For this reason, the preferred choice is the attribution of the total impacts according to system expansion. That is, the FishOil was set as the main co-product of the LimoFish and a credit was given for avoiding the production of a traditional fertilizer with the same characteristics. Specifically, according to Muscolo et al. (2022) [24], 1 kg of AnFert was assumed to replace 1 kg of NPK (ratio 1:1).

5.3 Sensitivity and uncertainty analysis

The sensitivity analysis was performed to test the robustness of the model created, enabling identification and quantification of the influence of certain parameters onto the environmental impact of the entire system [52]. Uncertainty evaluation was performed for the midpoint impact categories results. As discussed above, the

LCA model for the LabS was filled with primary data to ensure greater reliability of the outcomes. On the other hand, data of the SUp scenario were retrieved by the literature [37]. For this reason, for a quantitative determination of uncertainties associated to each LCI parameter we referred to the data quality pedigree matrix [52], a method which is widely employed [53][54][55][56][57] and recommended by the European Commission to assess the quality data in the Product Environmental Footprint and Organization Environmental Footprint [58].

The pedigree matrix (Table 5.12) lists the indicator scores assigned to each parameter and the related geometric standard deviation used in uncertainty analysis. In general, the scores associated to temporal, geographical and technological correlation were always estimated as the most optimistic situation (U value in the pedigree matrix = 1), while for reliability and completeness more severe scores were selected given the application of more assumptions (especially in case of SUp).

A Monte Carlo simulation with 10,000 runs was also carried out to determine how the intrinsic variability of the parameters and the quality of the data used in the modelling may affect the outcomes.

Table 5.12 Pedigree matrix.

	Trasport		Electricity LabS		Electricity SUp		Heat SUp		Limonene (reagent)	
U1 (reliability)	1,05	0,00	1,1	0,01	1,2	0,03	1,2	0,03	1	0,00
U2 (completeness)	1	0,00	1	0,00	1,2	0,03	1,2	0,03	1,05	0,00
U3 (temporal c.)	1	0,00	1	0,00	1	0,00	1	0,00	1,03	0,00
U4 (geographical c.)	1	0,00	1	0,00	1	0,00	1	0,00	1,05	0,00
U5 (technological c.)	1	0,00	1	0,00	1	0,00	1	0,00	1,05	0,00
Ub	2	0,48	1,05	0,00	1,05	0,00	1,05	0,00	1,5	0,16
SD		2,00		1,11		1,30		1,30		1,51

	Limonene (emission)		Biogas production		Electricity production		APK production	
U1 (reliability)	1,05	0,00	1	0,00	1,05	0,00	1,05	0,00
U2 (completeness)	1	0,00	1	0,00	1,02	0,00	1	0,00
U3 (temporal c.)	1	0,00	1	0,00	1	0,00	1	0,00
U4 (geographical c.)	1	0,00	1	0,00	1,001	0,00	1	0,00
U5 (technological c.)	1	0,00	1	0,00	1,05	0,00	1	0,00
Ub	1,5	0,16	1,05	0,00	1,05	0,00	1,05	0,00
SD		1,50		1,05		1,09		1,07

5.4 IMPACT World+: Midpoint analysis

Results are reported in form of contribution analysis for all the examined categories (Figure 5.2) and in form of environmental comparison for only climate change, long term (CChl) and freshwater eutrophication (FEu) categories (Figure 5.3). The selection is due to two main reasons. First, aligning to recent findings [53][48], CChl turned out to be the more contributing category to the single score. Second, the contribution analysis performed for all the impact categories (Figure 5.2) showed very similar outcomes for all the categories with the exception of the FEu. In particular, the contribution analysis proposed in Figure 5.2 allows to determine the specific contribution of each flow (e.g., electricity) or process (e.g., extraction) with respect to a specific environmental category by its colour intensity (the more intense is the color, the higher is the contribution of the flow or process on the impact category). CChl was accordingly assumed to be representative for the "CChl cluster", consisting of 17 categories (CChl, CChs, FAc, FEc, FNeu, HCT, HnCT, IRa, MEu, LOb, LTb, MRu, OLd, PMF, POf, TAc, WSc). Figure 5.3 shows the results for climate change, long term (CChl) and freshwater eutrophication (FEu) categories. For the sake of completeness, the outcomes of full and detailed LCIA were reported in the Table 5.13, 5.14, 5.15, 5.16.

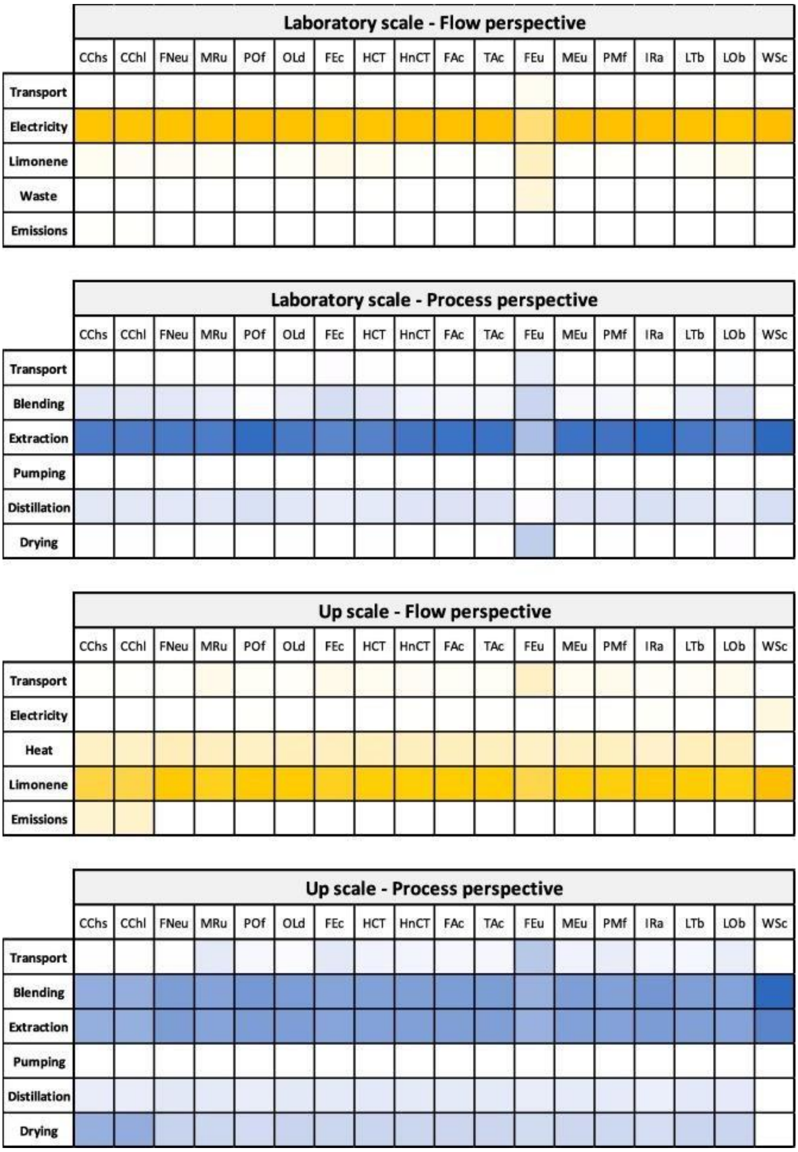

Figure 5.2 Contribution analysis of the LimoFish process referred to all the 18 impact categories examined representing (a) LabS, flow perspective; (b) LabS, process perspective; (c) SUp, flow perspective; (d) SUp, process perspective.

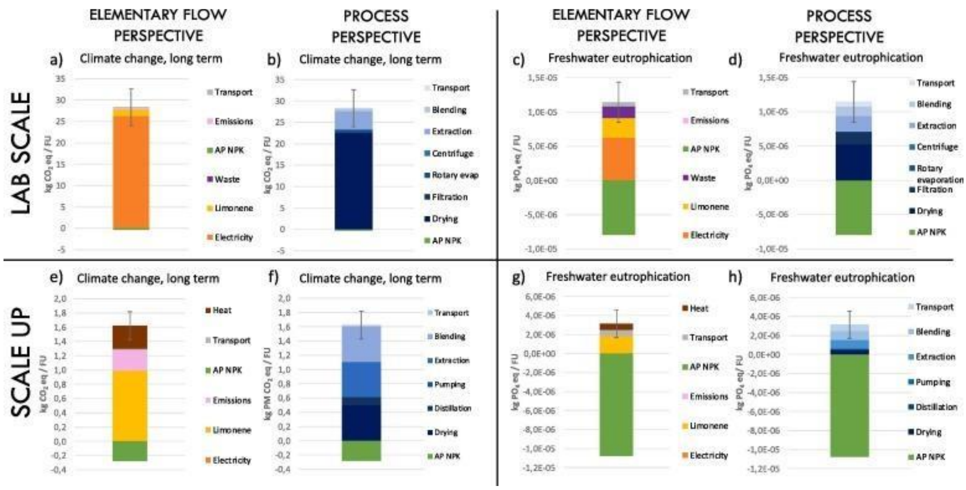

Figure 5.3 Environmental impacts of the production system. (a) CChl, LabS, elementary flow perspective; (b) CChl, LabS, process perspective; (c) FEu, LabS, elementary flow perspective; (d) FEu, LabS, process perspective; (e) CChl, SUp, elementary flow perspective.

Results are reported in two forms: elementary flow perspective (histograms on the left), and process perspective (histograms on the right). This visualization allows to extrapolate, to better split the information and to get the relevance and importance of each phase of the system. Error bars plotting uncertainty ranges are included.

The LabS results show that the main contributor to the environmental impacts is the electricity consumption (93 %, CChl). Indeed, this represents the most relevant flow also for the other categories (except FEu), going from a minimum of 90.0 % (LOb) to a maximum of 99.8 % (WSc). Concerning the FEu, electricity contributes “only” for 54.8 % of the total impact. The assumption of working at the 30 % of the maximum equipment power is therefore highly relevant for the calculation of the absolute impact values. In case of CChl, for instance, enhancing the power consumption to 50 % of the maximum, translates into an enhanced impact value from 29.3 kg of CO2 eq to 47.6 kg of CO2 eq. However, this assumption does not significantly affect the contribution evaluation, since the electricity remains the main contributing process. The avoided production of NPK compensates the direct impacts for ≥ 10 % only for the FEu (98.0 %) and LOb (22.5 %) categories. A very weak contribution is identified for the AnLeft transportation (always less than 1 % in the CChl cluster and 5.7 % in FEu) and for the use of virgin dlimonene, in agreement with its higher contribution to the FEu (24.7 %), while for the other categories it never reaches the 10 % of the direct impacts.

The process perspective identifies as main contributors the steps characterized by a high electricity consumption (drying). Quite different trends are observed in SUp, since optimizations associated with the upscaling of the process dramatically reduce the estimated impacts (by about 25 times in case of CChl). Such a reduction is justified also by the different power supply between the LabS and SUp equipment: while LabS is fed with electricity, which is, in the drying step, totally converted into heat, in SUp the most impacting steps (i.e., drying and distillation) are fed with heat directly generated from natural gas combustion. The reduced electricity requirement was reflected by a substantial decrease for both the CChl cluster and FEu values. The main contributor for all the examined categories, going from a minimum of 59.1 % (FEu) to a maximum of 74.3 % (IRa), turns out to be the input *d*-limonene, with nearly all (99 %) of the impacts associated to the virgin solvent production due to energy consumption. The CO_2 directly emitted into the atmosphere after the combustion of *d*-limonene affects only the CChs and CChl categories, participating to the direct impacts for the 16.5 % and 17.9 %, respectively. Inbound transportation, instead, generally contributes for less than 10 %, with the only exception of FEu, where it is responsible for 20.8 % of the total impact.

Electricity consumption, the main contributor in the LabS process, has its highest impacts in the water scarcity (11.1 %). The employment of the dry sludge as a fertilizer credits the overall process for the avoided production of an equivalent amount of NPK and it results in a netdiminution of the environmental impacts for 5 of the 18 analyzed categories (i.e., MRu, FEu, MEu, PMF and LOb). Concerning the process perspective, the highest contribution was observed for blending and extraction, due to the virgin *d*-limonene input.

Table 5.13 LCIA, LabS, flow perspective

	CChs (kg CO_2 eq)	**CChl** (kg CO_2 eq)	**FNeu** (MJ deprived)	**MRu** (kg deprived)	**POf** (kg NMVOC eq)	**OLd** (kg CFC-11 eq)	**FEc** (CTUe)	**HCT** (CTUh)	**HnCT** (CTUh)
Transport	2,0E-02	1,9E-02	2,9E-01	3,8E-04	6,7E-05	4,7E-09	1,9E+02	2,0E-09	1,7E-09
Electricity	2,7E+01	2,6E+01	4,8E+02	1,1E-01	2,2E-01	3,9E-06	2,4E+04	5,8E-07	1,3E-06
Heat	1,7E+00	1,5E+00	2,8E+01	4,9E-03	2,0E-03	1,8E-07	2,4E+03	4,1E-08	3,9E-08
Limonene	7,2E-02	7,2E-02	3,6E-01	4,3E-04	6,3E-05	8,7E-09	7,6E+00	2,8E-09	2,1E-09
Emissions	4,4E-01	4,4E-01	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00
AP NPK	-2,9E-01	-2,8E-01	-4,7E+00	-6,9E-03	-6,4E-04	-3,9E-08	-5,2E+01	-6,5E-09	-1,8E-08
Total	***2,9E+01***	***2,8E+01***	***5,1E+02***	***1,1E-01***	***2,2E-01***	***4,1E-06***	***2,7E+04***	***6,2E-07***	***1,3E-06***

	FAc (kg SO_2 eq)	**Tac** (kg SO_2 eq)	**FEu** (kg PO_4 eq)	**MEu** (kg N eq)	**PMf** (kg PM 2.5 eq)	**IRa** (Bq C-14 eq)	**LTb** (m^2/yr arable)	**LOb** (m^2/yr arable)	**WSc** (m^3 world eq)
Transport	1,3E-10	1,1E-07	6,5E-07	1,3E-06	6,0E-06	1,3E-01	4,2E-06	5,4E-04	1,2E-03
Electricity	1,7E-07	1,4E-04	6,3E-06	1,2E-03	4,2E-03	4,8E+02	2,7E-03	8,7E-02	1,1E+04
Heat	3,7E-09	3,0E-06	2,8E-06	2,5E-05	9,0E-05	3,4E+00	1,1E-04	8,5E-03	2,0E+01
Limonene	1,9E-10	1,6E-07	1,7E-06	2,0E-06	9,2E-06	1,5E-01	4,5E-06	7,0E-04	8,6E-03
Emissions	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00
AP NPK	-2,3E-09	-2,5E-06	-1,1E-05	-1,2E-04	-9,4E-05	-1,2E+00	-6,5E-05	-2,2E-02	-1,2E-01
Total	***1,7E-07***	***1,4E-04***	***2,2E-07***	***1,1E-03***	***4,2E-03***	***4,8E+02***	***2,7E-03***	***7,5E-02***	***1,1E+04***

Table 5.14 LCIA, LabS, process perspective.

	CChs (kg CO_2 eq)	**CChl** (kg CO_2 eq)	**FNeu** (MJ deprived)	**MRu** (kg deprived)	**POf** (kg NMVOC eq)	**OLd** (kg CFC-11 eq)	**FEc** (CTUe)	**HCT** (CTUh)	**HnCT** (CTUh)
Transport	2,0E-02	1,9E-02	2,9E-01	3,8E-04	6,7E-05	4,7E-09	1,9E+02	2,0E-09	1,7E-09
Blending	8,3E-01	7,5E-01	1,4E+01	2,5E-03	1,0E-03	9,1E-08	1,2E+03	2,0E-08	2,0E-08
Extraction	4,4E+00	4,2E+00	7,7E+01	1,7E-02	2,9E-02	6,0E-07	4,4E+03	9,6E-08	1,9E-07
Centrifuge	1,6E-02	1,5E-02	2,7E-01	6,1E-05	1,2E-04	2,2E-09	1,4E+01	3,3E-10	7,4E-10

Rotary evaporation	7,9E-01	7,6E-01	1,4E+01	3,1E-03	6,3E-03	1,1E-07	6,9E+02	1,7E-08	3,8E-08
Filtration	7,8E-02	7,7E-02	4,5E-01	4,5E-04	1,1E-04	9,5E-09	1,2E+01	3,0E-09	2,3E-09
Drying	2,3E+01	2,3E+01	4,0E+02	9,0E-02	1,8E-01	3,3E-06	2,0E+04	4,8E-07	1,1E-06
AP NPK	-2,9E-01	-2,8E-01	-4,7E+00	-6,9E-03	-6,4E-04	-3,9E-08	-5,2E+01	-6,5E-09	-1,8E-08
Total	***2,9E+01***	***2,8E+01***	***5,1E+02***	***1,1E-01***	***2,2E-01***	***4,1E-06***	***2,7E+04***	***6,2E-07***	***1,3E-06***

	FAc (kg SO_2 eq)	**Tac** (kg SO_2 eq)	**FEu** (kg PO_4 eq)	**MEu** (kg N eq)	**PMf** (kg PM 2.5 eq)	**IRa** (Bq C-14 eq)	**LTb** (m^2/yr arable)	**LOb** (m^2/yr arable)	**WSc** (m^3 world eq)
Transport	1,3E-10	1,1E-07	6,5E-07	1,3E-06	6,0E-06	1,3E-01	4,2E-06	5,4E-04	1,2E-03
Blending	1,8E-09	1,5E-06	1,4E-06	1,3E-05	4,5E-05	1,7E+00	5,5E-05	4,3E-03	1,0E+01
Extraction	2,4E-08	1,9E-05	2,2E-06	1,7E-04	5,9E-04	6,4E+01	4,0E-04	1,6E-02	1,5E+03
Centrifuge	9,5E-11	7,7E-08	3,6E-09	6,9E-07	2,4E-06	2,7E-01	1,5E-06	5,0E-05	6,5E+00
Rotary evaporation	4,8E-09	3,9E-06	1,8E-07	3,5E-05	1,2E-04	1,4E+01	7,7E-05	2,5E-03	3,3E+02
Filtration	2,2E-10	1,8E-07	1,7E-06	2,3E-06	1,0E-05	2,4E-01	5,0E-06	7,2E-04	2,2E+00
Drying	1,4E-07	1,1E-04	5,3E-06	1,0E-03	3,5E-03	4,0E+02	2,2E-03	7,3E-02	9,6E+03
AP NPK	-2,3E-09	-2,5E-06	-1,1E-05	-1,2E-04	-9,4E-05	-1,2E+00	-6,5E-05	-2,2E-02	-1,2E-01
Total	***1,7E-07***	***1,4E-04***	***2,2E-07***	***1,1E-03***	***4,2E-03***	***4,8E+02***	***2,7E-03***	***7,5E-02***	***1,1E+04***

Table 5.15 LCIA, SUp, flow perspective.

	CChs (kg CO_2 eq)	**CChl** (kg CO_2 eq)	**FNeu** (MJ deprived)	**MRu** (kg deprived)	**POf** (kg NMVOC eq)	**OLd** (kg CFC-11 eq)	**FEc** (CTUe)	**HCT** (CTUh)	**HnCT** (CTUh)
Transport	2,0E-02	1,9E-02	2,9E-01	3,8E-04	6,7E-05	4,7E-09	1,9E+02	2,0E-09	1,7E-09
Electricity	3,9E-03	3,8E-03	6,9E-02	1,5E-05	3,1E-05	5,6E-10	3,4E+00	8,2E-11	1,9E-10
Heat	3,6E-01	3,3E-01	5,9E+00	1,0E-03	3,6E-04	3,9E-08	5,3E+02	8,9E-09	8,2E-09
Limonene	1,1E+00	9,9E-01	1,8E+01	3,2E-03	1,3E-03	1,2E-07	1,6E+03	2,7E-08	2,6E-08
Emissions	2,9E-01	2,9E-01	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00
AP NPK	-2,9E-01	-2,8E-01	-4,7E+00	-6,9E-03	-6,4E-04	-3,9E-08	-5,2E+01	-6,5E-09	-1,8E-08
Total	***1,5E+00***	***1,3E+00***	***2,0E+01***	***-2,2E-03***	***1,1E-03***	***1,2E-07***	***2,2E+03***	***3,1E-08***	***1,7E-08***

	FAc (kg SO_2 eq)	**Tac** (kg SO_2 eq)	**FEu** (kg PO_4 eq)	**MEu** (kg N eq)	**PMf** (kg PM 2.5 eq)	**IRa** (Bq C-14 eq)	**LTb** (m^2/yr arable)	**LOb** (m^2/yr arable)	**WSc** (m^3 world eq)
Transport	1,3E-10	1,1E-07	6,5E-07	1,3E-06	6,0E-06	1,3E-01	4,2E-06	5,4E-04	1,2E-03
Electricity	2,4E-11	2,0E-08	9,0E-10	1,7E-07	6,0E-07	6,9E-02	3,8E-07	1,3E-05	1,6E+00
Heat	7,5E-10	6,1E-07	6,3E-07	5,1E-06	1,8E-05	5,6E-01	2,3E-05	1,9E-03	4,8E-03
Limonene	2,4E-09	1,9E-06	1,9E-06	1,6E-05	5,9E-05	2,2E+00	7,1E-05	5,6E-03	1,3E+01
Emissions	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00	0,0E+00
AP NPK	-2,3E-09	-2,5E-06	-1,1E-05	-1,2E-04	-9,4E-05	-1,2E+00	-6,5E-05	-2,2E-02	-1,2E-01
Total	***1,0E-09***	***1,5E-07***	***-8,1E-06***	***-9,6E-05***	***-1,0E-05***	***1,7E+00***	***3,4E-05***	***-1,4E-02***	***1,5E+01***

Table 5.16 LCIA, SUp, process perspective

	CChs (kg CO_2 eq)	**CChl** (kg CO_2 eq)	**FNeu** (MJ deprived)	**MRu** (kg deprived)	**POf** (kg NMVOC eq)	**OLd** (kg CFC-11 eq)	**FEc** (CTUe)	**HCT** (CTUh)	**HnCT** (CTUh)
Transport	2,0E-02	1,9E-02	2,9E-01	3,8E-04	6,7E-05	4,7E-09	1,9E+02	2,0E-09	1,7E-09
Blending	8,3E-01	7,5E-01	1,4E+01	2,5E-03	1,0E-03	9,1E-08	1,2E+03	2,0E-08	2,0E-08
Extraction	4,4E+00	4,2E+00	7,7E+01	1,7E-02	2,9E-02	6,0E-07	4,4E+03	9,6E-08	1,9E-07
Centrifuge	1,6E-02	1,5E-02	2,7E-01	6,1E-05	1,2E-04	2,2E-09	1,4E+01	3,3E-10	7,4E-10
Rotary evaporation	7,9E-01	7,6E-01	1,4E+01	3,1E-03	6,3E-03	1,1E-07	6,9E+02	1,7E-08	3,8E-08
Filtration	7,8E-02	7,7E-02	4,5E-01	4,5E-04	1,1E-04	9,5E-09	1,2E+01	3,0E-09	2,3E-09
Drying	2,3E+01	2,3E+01	4,0E+02	9,0E-02	1,8E-01	3,3E-06	2,0E+04	4,8E-07	1,1E-06
AP NPK	-2,9E-01	-2,8E-01	-4,7E+00	-6,9E-03	-6,4E-04	-3,9E-08	-5,2E+01	-6,5E-09	-1,8E-08
Tot	***29E+01***	***2,8E+01***	***5,1E+02***	***1,1E-01***	***2,2E-01***	***4,1E-06***	***2,7E+04***	***6,2E-07***	***1,3E-06***

	FAc (kg SO_2 eq)	**Tac** (kg SO_2 eq)	**FEu** (kg PO_4 eq)	**MEu** (kg N eq)	**PMf** (kg PM 2.5 eq)	**IRa** (Bq C-14 eq)	**LTb** (m^2/yr arable)	**LOb** (m^2/yr arable)	**WSc** (m^3 world eq)
Transport	1,3E-10	1,1E-07	6,5E-07	1,3E-06	6,0E-06	1,3E-01	4,2E-06	5,4E-04	1,2E-03
Blending	1,8E-09	1,5E-06	1,4E-06	1,3E-05	4,5E-05	1,7E+00	5,5E-05	4,3E-03	1,0E+01
Extraction	2,4E-08	1,9E-05	2,2E-06	1,7E-04	5,9E-04	6,4E+01	4,0E-04	1,6E-02	1,5E+03
Centrifuge	9,5E-11	7,7E-08	3,6E-09	6,9E-07	2,4E-06	2,7E-01	1,5E-06	5,0E-05	6,5E+00
Rotary evaporation	4,8E-09	3,9E-06	1,8E-07	3,5E-05	1,2E-04	1,4E+01	7,7E-05	2,5E-03	3,3E+02
Filtration	2,2E-10	1,8E-07	1,7E-06	2,3E-06	1,0E-05	2,4E-01	5,0E-06	7,2E-04	2,2E+00
Drying	1,4E-07	1,1E-04	5,3E-06	1,0E-03	3,5E-03	4,0E+02	2,2E-03	7,3E-02	9,6E+03
AP NPK	-2,3E-09	-2,5E-06	-1,1E-05	-1,2E-04	-9,4E-05	-1,2E+00	-6,5E-05	-2,2E-02	-1,2E-01
Total	***1,7E-07***	***1,4E-04***	***2,2E-07***	***1,1E-03***	***4,2E-03***	***4,8E+02***	***2,7E-03***	***7,5E-02***	***1,1E+04***

5.5 SUp sensitivity and uncertainty analysis

The contribution analysis highlighted two main variables which significantly affect the SUp results: the by-product valorization with AnFert replacing NPK, and the solvent production technology. A sensitivity analysis is applied to the i) by-product valorization: the solid residue is converted onsite via anaerobic digestion (AD) into biogas [22][59], and the latter gas is integrated with a cogeneration unit (CHP) able to convert the resulted biogas into electricity; and ii) the production technology of *d*-limonene in order to determine how results are impacted by theses variables.

Figure 5.4 displays the new valorization process. In this scenario (named SUp_{AD+CHP}), the drying process was not carried out to remove the presence of water, but principally to evaporate the impurities of nonrecycled *d*-limonene flow, which may inhibit the bacterial growth and compromise the biogas production [60]. The heat consumption and the environmental impacts associated to the AD are calculated through the "ecoinvent" database [42]. The environmental impacts of digestate produced in this phase (185 g/FU) were estimated according to the allocation proposed by Santiago et al. (2020) [20], in order to maintain the same evaluation criteria (90.3 %, TS1) of the rest of the study. This approach is considered very conservative, since the avoided production of material presumably replaceable by digestate is not considered. The volume of biogas produced (0.080 Nm^3/FU), conversely, is directed to the co-generator which theoretically allows the production of electricity (0.53 MJ_e/FU) and heat (0.76 MJ_t/ FU). A 100 % capture efficiency was assumed, with a 37 $\%\eta_e$ and 53 $\%\eta_t$ [42].

The electricity, assumed to be sold to the national grid, is included in the model as an avoided product. The heat produced was instead conveyed to the AD for self-consumption within the system. The heat excess was assumed to be dissipated into the atmosphere as heat waste. Environmental impacts of the co-generation and the credits associated to the avoided production of electricity are measured according to the ecoinvent database.

The environmental burdens associated to *d*-limonene utilization are calculated according to the inventory proposed by Santiago and coworkers (2020) [20]. However, as anticipated in the

LCIA, the production techniques may significantly alter the impacts associated to the solvent production. For this reason, the model was accordingly modified substituting the *d*-limonene produced via hydrodistillation, with that extracted via cold pressing. Since the prices of the three main products (*d*-limonene, biogas and digestate) of the process described by Santiago and coworkers (2020) [20] are very susceptible to market price volatility, the allocation criteria should also be considered as an interesting variable to investigate. Amid the two, the production technique (so-called SUp_{CP} scenario) was selected to perform the sensitivity analysis on the raw materials.

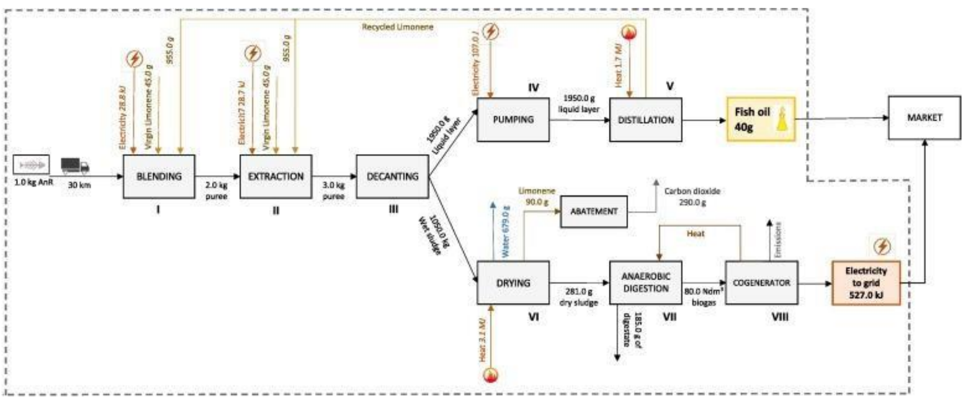

Figure 5.4. Alternative scenario. Anaerobic digestion and cogeneration processes as alternative to fertilizer production

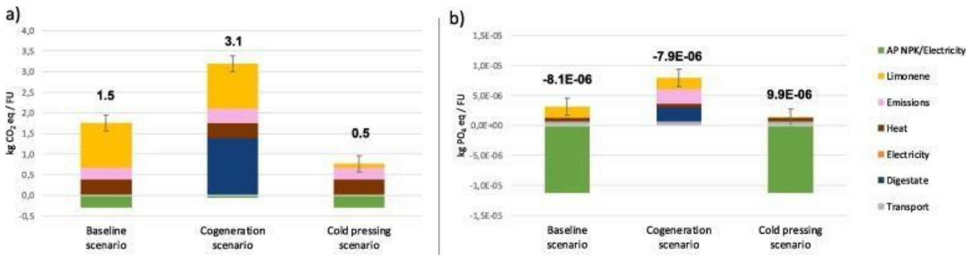

Figure 5.5 Sensitivity analysis considering baseline scenario (SUp) compared to SUp_{AD+CHP} and SUp_{CP}.

The analysis unveiled (Figure 5.5a and b) significant differences between the baseline scenario and the alternatives. Comparing the SUp with the SUp_{AD+CHP} scenario, for the CClh category the impacts increase of 1.6 kg of CO_2 eq/FU. Such an increase is due to the different byproducts management: the advantages originating from electricity production (-60.0 g of CO_2 eq/FU) are lower to those achievable by using the dry sludge as fertilizer (AnFert, − 310.0 g of CO_2 eq/FU). For this reason, despite the production of electricity from biogas obtained via AD is generally promoted by feed-in tariffs, in this case the alternative valorization route to organic fertilizer is shown to be advantageous from the environmental point of view. The SUp_{AD+CHP} scenario, furthermore, does not include the digestate management despite its appropriate valorization (e.g., amendment or fertilizer production) could credit the overall environmental performances. However, even supposing to apply a "zero burden" criteria to digestate, assuming that a proper valorization would compensate the environmental emissions, the final value of CChl would be 1.8 kg of CO_2 eq/FU, still higher than the 1.5 of the baseline scenario. The conversion of biogas into different energy carries, such as biomethane [61], could be in future investigated as further alternative.

On the other hand, the SUp_{CP} scenario shows higher impact reduction when compared with SUp. Employing the cold pressing technique instead of hydrodistillation, the impact associated to the raw material decreases from 1.1 (73 % of the direct impact) to 0.5 kg of CO_2 eq/FU (18 %) thanks to the lower heat consumption during virgin *d*-limonene manufacturing.

Lastly, the uncertainty analysis confirmed the good data quality, showing a relative standard deviation of 9 % in CChl and 10 % in FEu (uncertainty bars in Figure 5.3 and Figure 5.5) with respect to the absolute values. In general, the relative standard deviation associated to the results is lower or equal to 10 % in 8/18 categories (CChs, CChl, POf, FAc, Tac, FEu, MEu, PMf), lower or equal to 20 % in FNeu and higher than 20 % in the resting categories.

5.6 Conclusions

The LCA methodology was applied to evaluate the environmental performances of an innovative route to produce a new, whole fish oil (AnchoiOil) and a new organic fertilizer (AnchoiFert) starting from the anchovy fillet leftovers and citrus-derived *d*-limonene as a solvent and a stabilizing (antimicrobial) agent. The analysis of the process was carried out on laboratory and industrial scale (i.e., prospective LCA). The quantitative assessment enabled to evaluate the environmental impacts associated to each single flow and production step. The analysis suggests four clear outcomes.

First, the environmental impacts for the process at the laboratory scale are about 25 times higher than those estimated for the industrial process. Second, whereas on a laboratory scale electricity consumption is the dominant flow in terms of contribution to the final impacts, in the scaled-up process the production of virgin *d*-limonene is the most impactful stage. Third, the use of the solid by-product of AnchoiOil extraction as an organic fertilizer is significantly more sustainable than its employment as an input to anaerobic digestion with electricity production from biomethane combustion. Fourth, the production technology of *d*-limonene plays an essential role in the overall environmental impact evaluation, with cold pressing extraction leading to approximately 70 % lower direct environmental impacts. Clearly, electric power utilization (chiefly for *d*-limonene extraction but also for *d*-limonene recovery via evaporation under vacuum) is a crucial contributor to the environmental impact of the LimoFish process at both laboratory as well as at scale-up scale. In this respect, it is relevant the fact that a large fraction (or even all, in case of energy storage in Li-ion batteries) of the electricity required can be self-produced by bioeconomy companies willing to apply the process via roof-integrated solar photovoltaic (PV) modules. Today's, high-power, low-cost PV modules reliably produce on-site large amounts of electrical energy for over 30 years allowing companies to dramatically lower their environmental footprint, especially in sunny areas of the world such as those where anchovies are caught and processed.

While this study focused specifically on anchovies processing and valorization, the LimoFish process is general and can be applied to any fishery industry by-products. For instance, leftovers of the shrimp industry treated with *d*-limonene afford a valued marine oil rich in natural astaxanthin and a solid residue rich in chitin [23]. The approach of this study can thus be extended to the latter seafood leftovers to evaluate the overall environmental sustainability of the LimoFish process also in this case. LCA, in conclusion, confirms its value to evaluate the potential uptake of a new process and thus to support policy makers and stakeholders engaged with bioeconomy, including local communities for planning innovative and circular economy strategies for the valorization of biological residues.

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Chapter 6

Final conclusions and future perspectives

The global adoption of the Circular Economy model is crucial to mitigate the increasing environmental impact caused by the growing world population. The traditional Linear Economy approach, characterized by resource exploitation and waste accumulation, has led to significant environmental degradation and economic losses. Embracing the principles of the Circular Economy allows waste to be considered a valuable resource that can be used for the production of new materials and energy through proper treatment and valorization.

One sector with immense potential for circularity and sustainable resource recovery is the fisheries and aquaculture industry, which generates vast amounts of fish waste worldwide. Transforming this waste into valuable biomolecules and compounds, such as collagen, enzymes, bioactive peptides, and omega-3 fatty acids, can address both environmental concerns and economic losses associated with fish waste disposal. These compounds have diverse applications in fields like food, biotechnology, pharmaceuticals, and biofuel production.

To obtain high-quality compounds from fish discards, the extraction and purification techniques play a critical role. Prioritizing eco-friendly and green processes ensures the preservation of nutritional value and contributes to sustainable production practices. The wide range of applications for these compounds highlights the potential for significant cost recovery and economic growth in the fishing industry.

The Covid-19 pandemic has reshaped global market forecasts for some of these products, emphasizing the need for a more resilient and locally-focused economic model. The circular economy, with its emphasis on social well-being and environmental sustainability, emerges as a pivotal component of economic recovery plans.

The use of sustainability and green chemistry concepts in the fish industry-, particularly in utilizing fish by-products, presents an opportunity to reduce waste generation and environmental impact. Fish processing waste, like residues generated from anchovy fillet production, can be transformed into valuable bioactive compounds, including omega-3 lipids. The application of simple green chemistry processes using non-toxic solvents and moderate conditions can replace conventional extraction methods, ensuring both economic and environmental benefits.

Improving the extraction, purification, and stabilization methods of omega-3 from fish oil holds significant health, environmental, and economic importance. By producing better extracts and supplements, the efficacy of omega-3 products can be increased, benefiting consumers and society as a whole.

Anchovies, as one of the dominant small pelagic fish in the Indo-Pacific region, possess significant nutritional value, making them an excellent candidate for the extraction of valuable compounds. The anchovy fillet industry generates substantial biowaste that can be harnessed for the production of omega-3 rich fish oil and biomethane through anaerobic digestion.

Recent advancements in the extraction of fish oil from anchovy discards using *d*-limonene as a green extraction solvent at room temperature have shown promising results. *d*-limonene, derived from waste orange peel, offers advantages such as recyclability, antimicrobial properties, and the preservation of antioxidant compounds, which protect PUFAs from oxidation during extraction.

The utilization of anchovy discards for fish oil extraction, combined with green and circular economy principles, presents a sustainable and environmentally friendly method to produce high-quality omega-3 supplements. Scaling up this approach can reduce overfishing pressures and support a more balanced and efficient use of marine resources, contributing to better physical and mental health outcomes for global populations.

Additionally, the solid residue left after fish oil extraction and a subsequent drying step, known as solid anchovy sludge (SAS), proves to be, in a preliminary report, an ideal substrate for biogas production through anaerobic digestion. Despite the unbalanced carbon to nitrogen ratio typical of marine biowaste, the solid anchovy sludge exhibits a good methane yield (of about 280

$mL_{CH4}\cdot g_{VS}^{-1}$) and demonstrates stability during the digestion process. This makes it suitable for co-digestion with other carbon-rich biomass wastes and residues in modern biorefineries.

The success of this process lies in its ability to fully valorize anchovy fillet processing waste, closing the materials cycle and contributing to a more sustainable, circular economy.

In the next experiment presented, the utilization of anchovy sludge (AS), the solid residue left after fish oil extraction without any drying treatment, for biogas production, along with the prospect of co-digestion with other biomass wastes, creates a promising "blue-biorefinery" scenario, particularly in Mediterranean nations where significant amounts of biowaste are generated annually from small pelagic landings.

To address potential issues related to the presence of *d*-limonene in the substrate for the anaerobic digestion process, proper countermeasures are required. Particularly, in the following experiments, a co-digestion of anchovy sludge—used as a nitrogen supplement—and market waste (5% and 95% on a Total Solids basis) was performed.

This demonstrates the potential suitability of AS as a co-substrate for the anaerobic digestion of mainly carbonaceous feedstocks. However, the presence of *d*-limonene is an issue that requires proper countermeasures. The first is the optimization of the oil extraction process to reduce the residual solvent present in AS.

In addition, the results presented here, although preliminary, demonstrate how proper adaptation and the supplementation of GAC during anaerobic digestion can improve tolerance to *d*-limonene.

The utilization of biobased *d*-limonene as an extraction solvent for fish oil from anchovy fillet leftovers presents a groundbreaking circular economy process with multiple practical applications. The resulting solid residue, proved to be a powerful organic fertilizer, known as "AnchoisFert," that outperforms conventional organic (manure) and chemical (NPK) fertilizers. Rich in essential nutrients, such as proteins, organic carbon, flavonoids, magnesium, potassium, phosphate, and sulfate, AnchoisFert provides substantial benefits for crop growth and yield, exemplified by its significant positive impact on Tropea's red onion cultivation.

This newly discovered organic fertilizer not only closes the fishing material cycle for one of the most fished species across the seas but also marks a step towards sustainable and efficient resource management in both the fishing and agriculture industries. The circular economy process efficiently upgrades anchovy biowaste using *d*-limonene as a green solvent, leading to the production of valuable AnchoisOil fish oil, which can be commercialized in the omega-3 marine lipids market, and AnchoisFert, the dried fertilizer, sold directly to farmers for horticulture use.

AnchoisFert's exceptional performance as an organic fertilizer is due to its rich composition of nutrients and absence of antibiotics and antibiotic resistance genes. This eliminates the need for prolonged composting or further chemical, enzymatic, or thermal processing, making it a low-cost and environmentally-friendly alternative to other fish waste-derived fertilizers. Additionally, the residual *d*-limonene imparts a pleasant fragrance and antibacterial properties to AnchoisFert, minimizing the formation of rotting fish odor.

The successful proof of concept on Tropea's red onion, a highly valuable horticulture crop, showcases the potential practical utilization of AnchoisFert in other valued horticulture crops as well.

Moreover, the analysis of amino acids present in AnchoisFert further confirms its exceptional fertilization properties, with the presence of essential, quasi-essential, and nonessential amino acids playing a vital role in plant growth and metabolism. This supports the effectiveness of the circular economy LimoFish process in recovering valuable amino acids that would otherwise be lost in the environment, further enhancing the sustainability of anchovy fishing, processing, and consumption.

The prospective life cycle assessment (LCA) models developed and applied for the LimoFish process, which produces fish oil AnchoiOil, organic fertilizer AnchoisFert, or biogas through anaerobic digestion from anchovy fillet leftovers using *d*-limonene as a solvent, demonstrate the environmental benefits and sustainability of this innovative approach.

The analysis reveals that the environmental impacts of the LimoFish process are significantly lower at the industrial scale compared to the laboratory scale, indicating the efficiency and scalability of the process. Electricity consumption and the production of virgin *d*-limonene are identified as major contributors to the environmental impacts, highlighting the importance of optimizing these stages for a more sustainable outcome.

The utilization of the solid by-product as organic fertilizer proves to be a more sustainable option than using it for anaerobic digestion with electricity production from biomethane combustion. This finding emphasizes the value of circular economy principles in the fishing industry, where waste materials are transformed into valuable resources.

Additionally, the production technology of *d*-limonene plays a crucial role in the overall environmental impact evaluation, with cold pressing extraction showing a significant reduction in direct environmental impacts. The study also suggests that bioeconomy companies could further reduce their environmental footprint by self-producing a large fraction of the required electricity through on-site solar photovoltaic (PV) modules.

The general applicability of the LimoFish process to other fishery industry by-products opens up opportunities to extend the approach and evaluate the environmental sustainability of similar valorization strategies for other seafood leftovers.

Overall, the LCA approach employed in this study reinforces the value of the LimoFish process and supports policymakers and stakeholders in adopting innovative and circular economy strategies for the sustainable utilization of biological residues in the bioeconomy. This research provides insights into the potential environmental benefits of the process and contributes to the development of more sustainable practices in the fishing and bioeconomy sectors.

The future prospects for the LimoFish process and its associated products, such as AnchoiOil, AnchoisFert, and biogas, appear promising and hold significant potential for various industries and the environment.

1. Expansion of Circular Economy Principles: The LimoFish process exemplifies the circular economy approach, where fish byproducts are transformed into valuable resources like fish oil, organic fertilizer, and biogas. In the future, this circular model is likely to gain wider adoption in the fishing industry and beyond, leading to reduced waste generation, enhanced resource efficiency, and increased sustainability.
2. Enhanced Sustainable Fisheries: By utilizing fish byproducts for valuable applications, the LimoFish process can contribute to sustainable fisheries management. It helps

optimize the utilization of the entire fish and reduces pressure on fish stocks. This approach may encourage responsible fishing practices and support the preservation of marine ecosystems.

3. Advancements in Green Chemistry: The success of the LimoFish process is attributed to the use of biobased limonene as a green extraction solvent. Future advancements in green chemistry and solvent technologies may lead to even more environmentally friendly and efficient extraction methods for other valuable compounds from various biomaterials.
4. Diversification of Organic Fertilizers: The AnchoisFert organic fertilizer derived from anchovy fillet leftovers has shown exceptional performance in promoting crop growth. In the future, this could lead to the development of new organic fertilizers from other seafood leftovers or agricultural byproducts, offering sustainable alternatives to traditional chemical fertilizers.
5. Integration of Renewable Energy Sources: The LimoFish process relies on electricity consumption, particularly in the production of *d*-limonene. The integration of renewable energy sources, like solar photovoltaic modules, for on-site electricity production can further enhance the sustainability of the process and reduce its environmental footprint.

6. Sustainable Biogas Production: The anaerobic digestion of the solid residue from fish oil extraction for biogas production presents an additional avenue for sustainability. As the demand for renewable energy increases, biogas produced from fish processing waste may become a viable alternative to conventional fossil fuels.
7. Commercialization and Market Penetration: As the LimoFish process continues to prove its environmental benefits and economic viability, it is likely to attract interest from industries, governments, and consumers. This could lead to increased commercialization and market penetration of AnchoiOil, AnchoisFert, and other products derived from the process.
8. Regulatory and Policy Support: With growing concerns over environmental sustainability and resource conservation, regulatory bodies and policymakers may support and incentivize the adoption of circular economy practices like the LimoFish process. This could include providing funding, tax incentives, or certification programs to encourage businesses to implement such strategies.

The findings of this doctoral research carry profound implications for a diverse array of stakeholders engaged in environmental management, fisheries, and sustainable development. Governmental bodies and policymakers have a unique opportunity to strategically align policies with the tenets of the circular economy, contributing substantially to the realization of international commitments such as the United Nations' Agenda 2030. The introduction of innovative processes, notably the utilization of d-limonene in a closed-loop system for the valorization of fish waste, not only signifies a breakthrough in waste management but also opens avenues for regulatory frameworks that promote and incentivize sustainable practices within the fishing industry.

Industry stakeholders, especially those involved in fish processing and oil extraction, stand to gain significantly from the deployment of efficient and eco-friendly methodologies delineated in this research. The valorization of fish processing waste emerges not just as an economically viable prospect but also positions these sectors as trailblazers in the realm of sustainable resource management. The incorporation of life cycle assessment models, both at laboratory and industrial scales, offers invaluable insights into the environmental performance of the proposed methods, empowering industry leaders to make judicious decisions aimed at diminishing their ecological footprint.

Environmental advocacy groups and non-governmental organizations dedicated to sustainability find in this research a beacon of possibility. The successful integration of circular economy practices in the valorization of fish processing waste serves as a tangible solution to address the environmental challenges ingrained in traditional linear models. The utilization of anchovy fillet leftovers to extract omega-3-rich natural oil, coupled with the responsible utilization of by-products like Anchovy sludge, underscores the transformative potential within the fishing industry.

Educational institutions and researchers within the realms of environmental engineering and circular economy stand to gain valuable insights from the methodological advancements unveiled in this thesis. The introduction of d-limonene, sourced from citrus fruits, as an edible solvent within a closed-loop system not only exemplifies scientific innovation but also emphasizes the pivotal role of interdisciplinary collaboration in tackling intricate environmental issues.

In essence, this doctoral thesis, firmly rooted in the principles of the circular economy and sustainable development, transcends academic discourse. By seamlessly bridging the gap between theory and practical application, it furnishes actionable insights and concrete solutions for stakeholders spanning diverse sectors. Through collaborative efforts and strategic implementation, the envisioned sustainable future advocated by this research can be actualized, nurturing resilience, responsibility, and regeneration within the global fishing industry.

In conclusion, the LimoFish process shows great promise in contributing to a more sustainable fishing industry, reducing waste, and maximizing the value of fish byproducts. The potential applications of AnchoiOil, AnchoisFert, and biogas extend beyond the fishing industry, with possibilities for agricultural, energy, and environmental sectors. Future developments in technology, renewable energy, and supportive policies are likely to further enhance the impact and widespread adoption of this innovative and circular approach.

Other activities

Scientific production

- Paone, E.; Fazzino, F.; **Pizzone, D.M.**; Scurria, A.; Pagliaro, M.; Ciriminna, R.; Calabrò, P.S. **Towards the Anchovy Biorefinery: Biogas Production from Anchovy Processing Waste After Fish Oil Extraction with Biobased Limonene**. Sustainability 2021, 13(5), 2428.

- Pagliaro, M.; **Pizzone, D.M.**; Scurria, A.; Lino, C.; Paone, E.; Mauriello, F.; Ciriminna, R. Sustainably Sourced Olive **Polyphenols and Omega-3 Marine Lipids: A Synergy Fostering Public Health.** ACS Food Science & Technology 2021-03-19.

- Pagliaro, M.; Lino, C.; **Pizzone, D. M.**; Mauriello F.; Russo M., Muscolo, A.; Ciriminna, R.; Avellone, G. (2022). **Amino acid analysis in new organic fertilizer AnchoisFert**. 10.13140/RG.2.2.12056.67847.

- Ciriminna, R.; Scurria, A.; **Pizzone, D. M.,** Calabrò, P.S.; Muscolo, A.; Mauriello, F.; Pagliaro, M. (2021). **Economic and Technical Feasibility of AnchoisFert Organic Fertilizer Production.** 10.20944/preprints202111.0451.v1.

- Arfelli, F.; **Pizzone, D. M.**; Cespi, D.; Ciacci, L.; Ciriminna, R.; Calabrò, P.S.; Pagliaro, M.; Mauriello, F.; Passarini, F. (2022). **Life cycle assessment of the LimoFish process for the full valorization of anchovy fillet leftovers.** 10.26434/chemrxiv-2022-6c3fd.

- Angellotti, G.; **Pizzone, D. M.**; Pagliaro, M.; Avellone, G.; Lino, C.; Mauriello, F.; Ciriminna, R. (2023). **High stability of AnchoisOil extracted with limonene from anchovy fillet leftovers**. 10.13140/rg.2.2.21138.43202/1.

Attendance at conferences

- XII Congresso AICIng, Reggio Calabria 5-8 Settembre 2021. Poster: "*Valorizzazione degli scarti di lavorazione delle acciughe*"; authors: **Daniela Maria Pizzone**, Filippo Fazzino, Emilia Paone.

- Co-author Poster: "*Biogas production from anchovies residues after nutraceuticals extraction: first results*"; Filippo Fazzino, **Daniela Maria Pizzone**, Emilia Paone, Antonino Scurria, Rosaria Ciriminna, Paolo S. Calabrò. XI International Symposium On Environmental Engineering, Torino 29 Giugno-2 Luglio 2021.

- Workshop "I Giovani e la Chimica in Abruzzo", 6 Luglio 2021. "*Biogas production from anchovies residues after nutraceuticals extraction*"; **Daniela Maria Pizzone**, Filippo Fazzino, Emilia Paone, Antonino Scurria, Rosaria Ciriminna, Paolo S. Calabrò.

- XII Congresso SCICaSi 2022 Reggio Calabria 1-2 Dicembre 2022. Poster: "*Life cycle assessment of the limofish process for the full valorization of anchovy fillet leftovers*"; authors: **Daniela Maria Pizzone**, Francesco Arfelli, Daniele Cespi, Luca Ciacci, Rosaria Ciriminna, Paolo Salvatore Calabrò, Mario Pagliaro, Francesco Mauriello, Fabrizio Passarini.