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ATR-MIR and chemometric comparative studies on fish from different environments

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ABSTRACT

Fish is recognized for its elevated nutritional value, which is attributed to its substantial protein and fat content, among other constituents. The objective of this study is to explore the feasibility of utilizing ATR-MIR spectroscopy as a substitute for conventional techniques in determining the attributes of fish, particularly the fat and protein composition. Additionally, chemometric tools are employed in combination with ATR-MIR spectroscopy to ascertain the origin and storage conditions of the samples. The study is founded on fish sample wild, framed as well as fresh and frozen purchased from multiple markets around Venice.

Keywords: Fish, fat, protein, MIR spectroscopy, chemometrics, and PCA.

1. INTRODUCTION

The nutritional value of fish and fish products has led to a significant increase in consumer demand over the past few decades. The escalation of global commercial transactions and import/export operations contributed to a surge in sanitary hazards and commercial deceit, which are deeply linked to fish and seafood's perishable nature and economic significance. Spectroscopic techniques have gained increasing importance in the investigation of food composition during the past few decades. The application of mid-IR spectroscopy has been utilized as a rapid and precise technique for the analysis of multicomponent fish meat through FTIR(Fourier-Transform Infrared Spectoscopy). This technique has been employed to detect spoiled or contaminated meat products, verify the production method, specifically the type of breeding animals, and detect instances of meat adulteration, as previously demonstrated by Karoui (2010). In Al-Jowder(1997), the utilization of Principal Component Analysis (PCA) as a means of differentiation among unprocessed minced chicken, pork, and turkey meats are suggested. The protein and fat content are two parameters that are typically monitored throughout the meat supply chain. According to Elvingson's (2008) explain the process entails utilizing an alkaline detergent to dissolve fish meat with the aid of a blender, creating a suspension, and analyzing the obtained samples employing infrared spectroscopy.The research presented here includes an examination of three distinct species of fish: sardines, sea bream, and sea bass. These can be observed ubiquitously across the Mediterranean region and the eastern Atlantic. This research employs mid-infrared spectroscopy as an approach to investigate the characteristics of farmed, semi-farmed, and wild fish. The aim of this study is to investigate the feasibility of utilizing MIR spectral features of fat and protein to distinguish between fresh, frozen, and thawed food products through a rapid and facile approach. Furthermore, this study will address the techniques employed to establish correlations between the spectroscopic mid-infrared (MIR) data of diverse fish species and their constituent components. This study aims to enhance the advancement of reliable and practical techniques for examining specific technological and nutritional characteristics of these fish and to establish a mechanism for straightforward and uncomplicated differentiation among species of varying sources (semi-cultivated, cultivated, or wild). This is very helpful and useful activities for deterrence of fraudulent.

2. BACKGROUND

2.1 Global importance of fish

The consumption of seafood, specifically fish, holds significant importance in ensuring global food and nutrition security (FNS). According to Béné C et al. (2016) and FAO (2018), this is a result of its status as a primary and nutrient-rich animal source, particularly for many middle- and low-income countries (LMICs). Conversely, in nations with high incomes, there is an increasing recognition of the diverse nutritional and potentially health-enhancing attributes associated with the consumption of fish (Kawarazuka N. et al., 2011). Numerous epidemiological investigations have evaluated the positive impacts of fish consumption on various health outcomes(NSCFS (VKM) 2011; NSCFS (VKM) 2014). Food and Agriculture Organisation (FAO) in 2014, fish constitutes a substantial protein source, contributing to nearly 17% of the global protein intake. The depletion of fish resources resulting from excessive consumption has led to a reduction in their availability. As a result, aquaculture has emerged as the only viable means of satisfying the growing need for fish protein in the future.NSCFS (VKM) in 2011 and 2014, the main cause of excessive consumption has shown the significant levels of long-chain omega-3 polyunsaturated fatty acids (n-3 LCPUFA), specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Moreover, LCPUFA has been associated with improved fetal health, such as increased birth weight, a reduced probability of preterm delivery, and enhanced neurodevelopment, as reported by Middleton et al. (2018). Aquaculture is a noteworthy component of the industries within the global food economy that is experiencing accelerated expansion beyond initial projections. According to the Food and Agriculture Organisation (FAO), there has been an 8.5% rise in aquaculture production over the last 25 years, which has contributed to approximately 50% of the total fish production intended for human consumption (FAO, 2014). Based on data from UNEP (2001), fish plays a significant role in providing animal-based protein to the protein intake of approximately 950 million individuals globally.

2.2 Nutrition and Food Security: An Essential Necessity

Food insecurity is defined as the failure to meet the Food and Nutrition Security (FNS) standards, as stated by the Food and Agriculture Organisation (FAO) in 2015. As per the definition provided by the United States Department of Agriculture (USDA), food insecurity is characterized by the restricted or ambiguous accessibility of nutritionally safe and sufficient food or the limited and uncertain ability to procure acceptable food in socially appropriate manners (G. Bickel et al., 2000; p. 6). This definition has been acknowledged by the Food and Agriculture Organisation (FAO) in particular (FAO 2015, p. 53). Food insecurity is a complex issue that arises from various factors, both temporary and long-term in nature. Temporary factors include climate change, harsh weather, and conflicts, while long-term factors encompass low income and reduced food consumption due to illness. These factors have been extensively studied by organizations such as the Food and Agriculture Organisation (FAO) and the United Nations Children's Fund (UNICEF). The lack of access to adequate food and nutrition is a violation of a basic human entitlement and can lead to various health and nutritional complications as well as developmental issues for the entire population, as stated by G. Bickel et al. (2000).

2.3 Consequences of inadequate food and Nutrition

The development of various malnutrition types, including undernutrition, overnutrition, and micronutrient deficiencies, is contingent upon the extent of food and nutrition insecurity, as per the Food and Agriculture Organisation's reports in 2018 and 2015 (p. 53). The lack of adequate healthcare services, contaminated drinking water, inadequate sanitation, and the consequent prevalence of various diseases, malnutrition, and food insecurity can result in an incapacity to fulfill dietary requirements due to insufficient and substandard food consumption (FAO 2018; UNICEF 1991). Insufficient food intake over a prolonged duration, commonly referred to as "chronic hunger" (FAO 2018; WHO 2019), can lead to a range such as anaemia in women of childbearing age, stunting and wasting in children, as well as various forms of micronutrient deficiencies and other undernourishment-related disorders. The term "hidden hunger" is often used to describe deficiencies due to the absence of apparent physical symptoms (FAO 2018; Abeywickrama HM et al., 2018). This condition is estimated to affect approximately 2 billion individuals worldwide (WHO, 2014). The most widespread and persistent micronutrient deficiencies affecting global population health are those pertaining to iron, vitamin A, iodine, folate, and zinc. The aforementioned deficiencies have been found to be associated with an increased likelihood of both mortality and morbidity, as reported by Bailey and colleagues (2015) and UNICEF (2018). According to the Food and Agriculture Organisation (FAO), approximately 821 million individuals, which equates to one-ninth of the global population, were deemed to have encountered food insecurity for three consecutive years as of 2017. According to the FAO report of 2018, it is expected that the quantity will experience a further increase. The correlation between moderate food insecurity and the development of overweight and obesity, as well as nutritional deficiencies, can be exemplified by the prevalence of a diet that is high in calories but deficient in micronutrients. This association was documented by the Food and Agriculture Organisation in (FAO 2018). The prevalence of malnutrition, including various forms, is currently pervasive across multiple countries, regions, households, and also throughout an individual's lifespan. The coexistence of malnutrition, obesity, and non-communicable diseases (NCDs) is commonly known as the "double burden of malnutrition," while the combination of vitamin deficiencies, obesity, and NCDs is referred to as the "triple burden of undernutrition" in academic literature (John Ingram (IFPRI) 2018; Pinstrup-Andersen P. 2007). Insufficient accessibility to nutrient-dense food, leading to food and nutrition insecurity, is a contributing factor to both undernourishment and obesity, as per the Food and Agriculture Organisation's report in 2018.

2.4 Fish meat components and nutritional characteristics

The chemical composition of many fish species has been the subject of a great deal of research in the past, the majority of which has been accomplished by Atwater (1888), who is credited with doing the most extensive of these studies. His work comprised about 53 species of American food fishes and since then became an important source of information. Although there are variations in nutrient composition among the marine fish species.(EFSA 2014; J. Murray & et al., 2001; Larsen R. et al., 2011). The approximate composition of fish muscle tissue, commonly referred to as fillet, comprises four primary natural characteristics, namely water, proteins, lipids, and ash This information has been documented by J. Murray et al., (2001) and Paine RT (1971). According to Paine's research conducted in 1971, there is limited knowledge regarding the chemical

composition of fish bones and its potential implications on health and nutrition, despite recent findings indicating that bone mass comprises varying amounts of collagen-associated amino acids and various lipids. Fish is considered to be a valuable source of marine n-3 LCPUFA, high-quality animal protein, and various vitamins and minerals such as zinc, selenium, iodine, vitamin A, vitamin B12, and vitamin D. This information has been supported by EFSA in 2014, NSCFS (VKM) in 2018, Lisbeth Dahl TB et al. in 2006, and EFSA in 2005. In addition, it is worth noting that the bone constituent, comprising approximately 10-15% of the overall fish biomass, is rich in minerals such as calcium and phosphorus, as reported by the Food and Agriculture Organisation in 2018, Toppe et al. in 2007, and Larsen et al. in 2000.

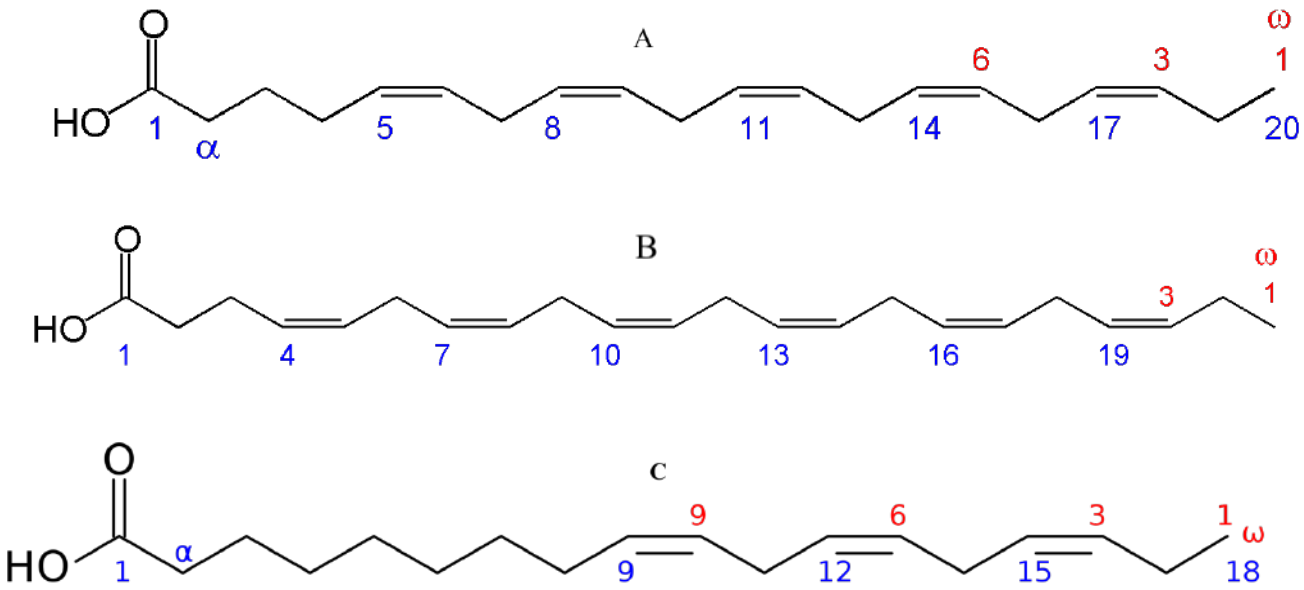
The purpose of their investigation was to evaluate seasonal variations that affect the chemical composition of fish nutrition. Subsequently, Mannan(1961) published a series of papers concerning the biochemical constitution of Canadian fish. Reviewed the chemistry of fish in his two books entitled Chemical Biology of Fish and is therefore considered the 'Father of Fish Chemistry'. Stansby(1962) attempted to illustrate the chemical composition of fish and stated that the approximate composition of fish is taken basis on: The dry weight basis is utilized for analysis, The wet weight basis is also employed for analysis, The carcass composition is determined by analyzing the whole fish, Analysis of an edible portion, such as muscle tissue, bone, scales, etc. It has been shown that a distinct association exists between the moisture and fat amount besides protein and fat content. The degree of variation is most pronounced in the case of fat or oil content, up to factors as great as 300, whereas moisture, protein, and ash content display a comparatively lower level of discrepancy, approximately five times. On the basis of variation of protein and fat content in the muscles, Stansby and Olcott (1963) categorized the fish into five different groups, which are enlisted in Table.1

Table 1: Five categories of fish according to the protein and fat content.

Types	**Oil content**	**Protein content**
Low oil−high protein	Less than 5%	15%−20%
Medium oil−high protein	5%−15%	15%−20%
High oil−low protein	More than 15%	Less than 15%
Low oil−very high protein	Less than 5%	More than 20%
Low oil−low protein	Less than 5%	Lower than 15%

2.4.1 Fatty acid Composition

The composition of fatty acids in fish can be described as a mixture of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and large amounts of polyunsaturated fatty acids (PUFA) (EFSA 2005). Omega-3 fatty acids, which are indispensable components of the human diet, are classified as polyunsaturated fatty acids, featuring a double bond at the third carbon atom from the terminal end of the carbon chain. The Fatty acid includes several large compounds, namely eicosapentaenoic acid($C_{20}H_{30}O_2$)(Figure1-A), docosahexaenoic acid($C_{22}H_{34}O_2$)(Figure1-B),α-linolenic acid ($C_{18}H_{30}O_2$))(Figure1-C), stearidonic acid($C_{18}H_{28}O_2$) (Figure1-D), and docosapentaenoic acid($C_{22}H_{32}O_2$) (Figure1-E). The composition of fish oil is rather complex. Each of these substances exhibits unique biological effects. Fish exhibit a unique quantity of n-3 fatty acids owing to a natural food chain that involves phytoplankton that produce n-3 fatty acids, which are consumed by zooplankton that are subsequently consumed by fish (NSCFS (VKM) 2006). In addition, it has been reported that fatty and medium-fat fish contain higher levels of n-3 LCPUFA compared to lean fish, as stated by various authoritative sources such as FAO (2003), EFSA (2005), and NSCFS (VKM) (2006). According to Bhouri et al. (2010), fish muscle tissue exhibits a greater propensity for accumulating fatty acids in comparison to the liver. Research has demonstrated that essential fatty acids (FAs) of this nature possess potential benefits that may mitigate the risk of cardiovascular disease (Chin and Dart, 1995). The nutritional significance of fish, particularly with regard to docosahexaenoic acid (DHA), has been extensively explored in Khalili Tilami's 2018 review. Due to its crucial role in preserving adult brain function and promoting an infants' brain growth and development, Horrocks and Yeo (1999) noted that DHA has been the subject of much discussion of the potential health advantages associated with the ingestion of polyunsaturated fatty acids (PUFAs), specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have prompted a surge in attention towards the consumption and fortification of these fatty acids in commonly consumed food items (Mozaffarian and Rimm,2006;Pourashouri et al., 2014;Kaushik et al., 2014).



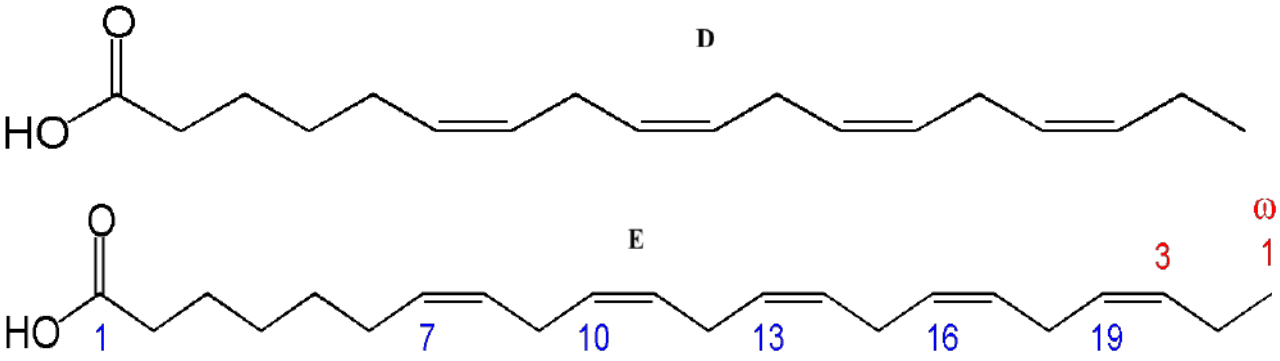


Figure 1: Chemical structure of omega-3 fatty acids. A: Eicosapentaenoic acid (EPA); B: Docosahexaenoic Acid (DHA); C: α-Linolenic Acid (ALA); D: Stearidonic Acid; E: Docosapentaenoic Acid (DPA).

2.4.2 Protein Content

Proteins are widely regarded as the most multifaceted biomolecules due to their composition of amino acids that are linked by a peptide bond. According to Pal et al. (2018), fish proteins exhibit a remarkably high concentration of amino acids and digestibility, ranging from 85% to 95%. The nutritional value of fish protein has been widely acknowledged in academic literature, with numerous studies highlighting its positive impact on human health (Khalili Tilami & Sampels, 2018). Its primary function involves the construction and restoration of muscular tissues, as well as the enhancement of blood composition and the immune system. According to Balami et al. (2019), immunoglobins, which are proteins, serve as a potent means of protecting against viral and bacterial infections, as well as assisting in the regulation of water balance and electrolyte systems in humans. Research on fish proteins is still in its early stages, but investigations have been conducted on their potential roles in addressing insulin resistance, obesity-related concerns, cancer development, osteoporosis, metabolic syndrome, and inflammation. According to Khalili Tilami and Sampels (2018), fish proteins, peptides, or hydrolysates have been found to have a noteworthy impact in many fish lipids. In accordance with Ryu et al. (2021), it is generally observed that the protein content in fish muscle ranges from 15% to 25%. The evaluation of fish meat texture and quality is a crucial aspect, wherein the protein content of fish is deemed highly significant and is acknowledged as the most superior protein source in developing nations (Palani et al., 2014). The protein requirement of fish is influenced by both the quantity and quality of fish, as higher protein content in the fish body necessitates a commensurate increase in protein for fish growth, and conversely. Similar to other organisms, fish necessitate proteins solely for obtaining the amino acids that constitute the novel proteins, and the nutritive efficacy of proteins is primarily contingent on the amino acid composition (Wilson, 2003).

2.4.3 Water Content

Water constitutes a significant proportion of the anatomical structure of all living beings, including aquatic organisms, and serves as a medium for the conveyance of diverse nutrients, the transfer of chemical energy, and numerous cytoplasmic reactions. The majority of fish species exhibit a moisture value that typically falls within the range of 60% to 80% (Aberoumand, 2014). More over, certain fish species have been observed to possess even higher moisture values, such as *Harpadon nehereus*, commonly known as the Bombay duck, which has been found to exhibit a moisture value of approximately 90% (Love, 1970). As stated by Jolaoso et al. (2016), the proportion of water content in a substance can serve as a reliable indicator of its corresponding levels of protein, energy, and lipid. *Septipinnataty* was found to possess low moisture and high-fat content, as reported by Ramaiyan et al. (1976). In accordance with Jacquot's (1961) findings, there is an inverse correlation between the moisture and fat content of fish. Specifically, the observed moisture levels were 68.6% in fatty fish, 77.2% in semi-fatty fish, and 81.8% in lean fish. These data confirm that an estimation of the energy, protein, and fat levels can be made based on knowledge of the relative quantity of water present in the fish (Ali et al.,2006). Dambergs' (1956)the moisture content of fish muscle was observed to increase as the fish matured and immature fish exhibited the lowest moisture content, which tended to increase as they matured. Several researchers have investigated the correlation among moisture, fat, and protein composition, as evidenced by the works of Memon et al. (2010), Njinkoue et al. (2016), Odoli et al. (2019), and Zhang et al. (2014). Chari (1948) and Jafri (1968) observed moisture content values exhibited a positive correlation with gonad maturation, with an increase in value occurring subsequent to spawning. In her research, Hanna (1985) investigated the correlation between age and the proximate composition of *Variola louti* muscles. The results indicated an inverse relationship between moisture content and fat content in the muscles. The observed differences in the proximate composition of fish in relation to age could potentially be attributed to spawning and migration, as noted by Ndome et al. (2010). The study conducted by Breck (2014) examined the correlation between body size and fish constituents. The findings revealed a robust relationship between protein mass and water, wherein the amount of water per unit of protein decreased with an increase in fish size. The presence of this type of relationship and its occurrence in various fish species have been attributed to biochemical or physiological factors. There exists a positive correlation between the quantity of water and ash mass, whereby the ratio of water to ash per unit decreases in fish of greater sizes.

2.4.4 Vitamin and Mineral Lipid Content

Fish is a nutrient-dense food source that contains a variety of essential vitamins and minerals, including but not limited to vitamin A, vitamin B12, vitamin D, iodine, selenium, and zinc. Regarding its quantitative aspect, they occupies the fourth position and exhibit a range of 0.5% to 5% in relation to the overall body mass of fish. According to Ndome et al. (2010), ash is typically recognized as a notable provider of nutrients for fish. According to Bano's (1977) findings, there exists a significant correlation between the ash content in fish bodies and their various conditions. The utilization of ash has been linked to the identification of minerals in fish and proved the most dependable approach to determine the mineral composition of fish. This is due to the fact that ash represents the complete inorganic or mineral content of the fish specimen. According to reports, the overall mineral composition found in the muscle tissue of aquatic organisms in their hydrated state typically falls within the range of 0.6% to 1.5% of the organism's total body mass. The

musculature and skeletal structures of fish are considered to be highly valuable sources of non-essential minerals, with a significant proportion of approximately 65% of these minerals being stored within the vertebrae (Njinkoue et al., 2016). According to Rahman et al. (2020), various factors, including but not limited to diet, species, and environmental variables such as temperature, seasons, salinity, and geographical location, are the primary contributors to the variability in mineral concentration observed in fish and shellfish. In addition, it has been observed that the composition of minerals and trace elements, which constitute the entirety of ash content, is influenced by various factors such as feeding patterns, migration patterns, and environmental conditions, even among organisms coexisting in the same habitat (Abdallah, 2007; Palani et al., 2014). The lipid content of fish is arguably the most dynamic component in its entirety. The biochemical composition of fish is subject to variation based on a variety of factors, including inter-species differences, geographic location, seasonal changes, ecosystem characteristics such as water temperature, salinity, pressure, dietary intake, and the fish's stage of maturity, sex, and reproductive cycle. This information has been documented by various sources, including J. Murray et al., (2001), EFSA (2005), H. H. Huss. (1995), FAO (1980), and IMR (2015). The sources cited in the text are FAO (2003) and Huss (1995). The deposition of triacylglycerol, a lipid species, exhibits heterogeneity across the fillet: its concentration increases from the tail region towards the head, while decreasing from the dorsal to ventral regions (J. Murray et al., 2001, EFSA 2005, and Bell JG et al., 1998). Moreover, substantial quantities are observed in the red muscular tissue and subcutaneous layer of the fish (Bell JG et al., 1998). According to Duran et al., (2010), minerals have a crucial function in preserving bodily processes, including the maintenance of acid-base equilibrium and the creation of haemoglobin. Additionally, they participate in the process of osmoregulation and contribute to the formation of bones and teeth. Furthermore, they function as catalysts in various enzyme-catalyzed or metabolic reactions, serving as either activators or inhibitors, as noted by Njinkoue et al., (2016). In addition to their nutritional and physiological roles, minerals are known to contribute to the flavor and texture of food (Ersoy & Celik, 2010).

2.5 The influence of provenance – wild/farmed

In recent times, aquaculture has emerged as a significant industry, offering superior-quality products that are environmentally advantageous and conducive to human well-being. The only way to cope with the danger of infection during the early stages of aquaculture's development was to use antibiotics, which were used in limited areas with certain diets and processes. Numerous research studies have demonstrated that farmed fish exhibit greater consistency in their nutritional and lipid compositions compared to their wild counterparts, resulting in higher levels of n-3 fatty acids (Testi S et al., 2006; Tasbozan O et al., 2016; Cahu C et al., 2004). Several academic studies have reported differences in the dietary composition of wild and farmed fish, as well as between freshwater and saltwater fish (Erdem et al.,2009), Alasalvar et al. (2002), Ravichandran et al. (2012), and Bhouri et al. (2010). In contrast to their wild counterparts, farmed fish are subjected to regulated conditions throughout their entire life cycle, spanning from fertilization to the point of slaughter. The fatty acid (FA) composition and other attributes of fish muscle can be influenced by various factors, including but not limited to environment and diet, which can be regulated throughout the lifespan of the fish (Henderson and Tocher 1987 and Ackman 1989). Each stage of the fish's life cycle, such as cultivation, rearing, and processing, can exert a substantial influence on the ultimate quality of the end product. Furthermore, disparities in lipid composition between cultivated and natural fish have been documented in previous studies (Vidal et al., 2014; Vidal et al., 2012). Fasolato et al. (2010), the discrimination of distinct wild and farmed sea and freshwater

fish species necessitates the integration of fatty acid and chemical composition analyses with carbon and nitrogen isotope ratio measurements. According to Saito et al. (1999). Fish is considered to be a highly beneficial source of omega-3 fatty acids, with its lipid content and composition being subject to variation based on factors such as intake, organism type, fertility, size, maturity level, water temperature, waterlogging, and weather conditions (Khalili Tilami, 2018; Grigoris, 2007). It has been observed that during periods of high feeding, there is an initial increase in protein content in the muscles, followed by a rapid increase in fat content. In contrast, it has been observed that a significant decrease in fat and protein content may occur during periods of starvation, spawning, migration, or when food is not readily available (Huss, 1988, 1995). According to Khalili Tilami and Sampels (2018), feed composition, as well as the rearing system and feeding schedule, have a significant impact on lipid content and fatty acid composition. In contrast, Morris (2001) posits that once fish are provided with diets containing sufficient quantities of all essential nutrients, the protein content in their bodies seems to be predetermined for each specific species, regardless of the amount of food or feeding schedule. According to Baker (2001), akin to proteins, the ash content quantity and mineral composition in fish are predetermined, although certain micronutrients may be influenced, potentially impacting the fish flesh's quality. Over the last decade, there has been a notable shift in the utilization of pelagic sources in aquafeeds due to their restricted availability and higher production cost, in favor of vegetative sources that offer lower costs. This trend has opened up the potential for greater incorporation of agricultural vegetation supplies in aquafeeds, as reported by Gatlin et al. (2007) and Naylor et al. (2009).

2.6 The Influence of Conservation

According to the literature, various factors like storage, transport, handling, and spoilage have a significant impact on the quality of fish (Khalili Tilami & Sampels, 2018). Upon the demise of the fish, circulation ceases, leading to a depletion of oxygen levels within the fish's body. Consequently, the synthesis of ATP is impeded as the electron transport chain and oxidative phosphorylation processes are rendered non-functional. In addition, the fish's body undergoes an anaerobic conversion of glucose, resulting in the accumulation of lactic acid and a decrease in pH levels. This decrease in pH levels hastens the process of protein denaturation and rigor mortis. Furthermore, the processing of fish, akin to the frozen stage, exerts a significant impact on the fish's composition, particularly with regard to the functional properties of its proteins. Certain illicit traders engage in the sale of fresh fish that has undergone the process of freezing and subsequent thawing as a means of augmenting their financial gains. Consequently, it is imperative to conduct research aimed at identifying a prompt and reliable technique for discriminating between fresh and frozen-thawed fish, as well as determining the duration for which the fish have been preserved. The nutritional value and sensory qualities of fish fillets tend to diminish over time during storage, freezing, or thawing procedures by Karoui et al., (2007). Karoui et al., (2017) have identified several processes that contribute to the deterioration of fish quality, including protein denaturation, fat oxidation, ice crystal formation, tissue damage, and alterations in enzyme activity. Consequently, the sensory and market values of Frozen fish are expected to decrease, as noted by Shan et al. (2018). At present, prognostications regarding the duration of fish storage are predominantly conducted through microbiological methodologies (Zhang L. et al., 2010). The procedure is complex and yields dependable outcomes; Moreover, protracted testing procedures are complex for implementation in online or large-scale settings, and are inadequate for meeting the requirements of expedient and non-invasive testing.

3. THE FISH SPECIES INVESTIGATED

3.1 Seabass

Dicentrarchus labrax, commonly known as the European Sea Bass(Figure 2), is predominantly distributed across marine, lagoon, and estuarine ecosystems characterized by the confluence of saline oceanic water and fresh water from the mainland. The fish species commonly referred to as Branzino, which is one of its Italian names, enjoys widespread usage.

Figure 2: Sea Bass (*Dicentrarchus labrax*).

This particular fish exhibits slender scales, a distinctly bifurcated tail fin, and a coloration ranging from silver-grey to blue(Figure 2). The average weight of a fully-grown European seabass is approximately 5 kilograms. Whitehead et al. (1986), the region in question spans from the western Mediterranean to the Black Sea, crossing the northeastern coast from Norway to Morocco(Figure 3). The demersal fish commonly known as wild seabass inhabits coastal waters at depths of up to 100 metres.Sea bass is commonly found in shallow waters with a variety of substrates and has been observed to frequently inhabit estuaries and occasionally migrate upstream in rivers. This is classified as euryhaline, meaning it is capable of surviving in various types of water, including both fresh and salt water.

Figure 3: The Major producer countries of Sea Bass(FAO 2006).

Additionally, it is categorized as eurythermic due to its ability to tolerate a broad range of water temperatures.It has been observed that juvenile fish tend to form schools, whereas adult fish exhibit less social behavior. The reproductive period spans from January to March, with an extension until June for populations inhabiting the Atlantic region. This species is a highly predatory organism

that consumes a diverse array of invertebrates, such as prawns, crabs, squids, and shellfish, in addition to small, schooling fish.

The European seabass farming industry is deemed profitable owing to the high demand and increasing price of this fish over the last decade. According to Alasalvar et al. (2002), European seabass possesses a desirable scent and is rich in nutrients. The cultural significance of sea bass was established in the 1960s, when it began to be cultivated in coastal lagoons and tidal reservoirs, notably in France and Italy. Initially, the production of salt in evaporation pans and marshlands situated along the coast was associated with pisciculture. In the low evaporation season, which spans from winter to spring, cohorts of fish inhabiting estuarine environments were captured and subsequently cultivated on farms. According to Morettia et al. (1999), the species under consideration is currently the primary and most significant fish that is being cultivated for industrial purposes in the Mediterranean Sea. In addition, the successful implementation of a mass-production culture technique for sea bass larvae during the 1970s facilitated the widespread cultivation of this species in Europe and Mediterranean Sea countries, thereby making it the first non-salmonid species to be extensively farmed in the region (FAO Linnaeus, 1758). The following table 2 explains the Characteristics of Sea Bass.

Table 2: Characteristics of Sea Bass (*Dicentrarchus labrax*) (FAO).

PARAMETER	**CHARACTERISTICS**
Temperature	5-28°C.
Habitat	Coastal water, about 100 meters deep.
Diseases in farming	Bacteria (vibriosis, photobacteriosis, myxobacteriosis, mycobacteriosis, epitheliocystis) and viruses (encephaloretinopathy)
Maturation	1-1.5 years.
Diet in the wild	Crabs, small fish, cuttlefish, and prawns.
Diet in farming	Fish meal (mostly manufactured feeds).
Juvenile phase	75 days.
Grow-out	1-1.5 years.
Distribution in the wild	North Atlantic from Norway and the British Isles southward to Morocco, the Canaries, and the Mediterranean and Black Seas.
Farming	France, Turkey, Egypt, Croatia, Greece, Spain, Italy, Croatia, Tunisia, Cyprus, and to some extent other Mediterranean countries.
Farming system	estuarine semi-intensive, sea-cage systems (the most common), or tank systems.

In 2010, China started the cultivation of Greek sea bass through importation. Various studies have indicated that European sea bass is a suitable species for high-density aquaculture due to its rapid growth and strong resistance to diseases (Zhang et al., 2014). Branzino is a nutritionally valuable food item due to its high protein content, significant presence of selenium, and abundance of omega-3 fatty acids. The European bass is a commonly served fish in restaurants and is known as sea bass in Ireland and the United Kingdom. In 2016, the average per capita consumption of seabass in the Mediterranean basin was 200 g. According to EUMOFA's report in 2019, Greece, Portugal, and Cyprus were the top three countries in terms of per week consumption of sea bass, with 796 g, 680 g, and 643 g, respectively. Spain and Italy followed closely behind, with 545 g and 513 g, respectively.

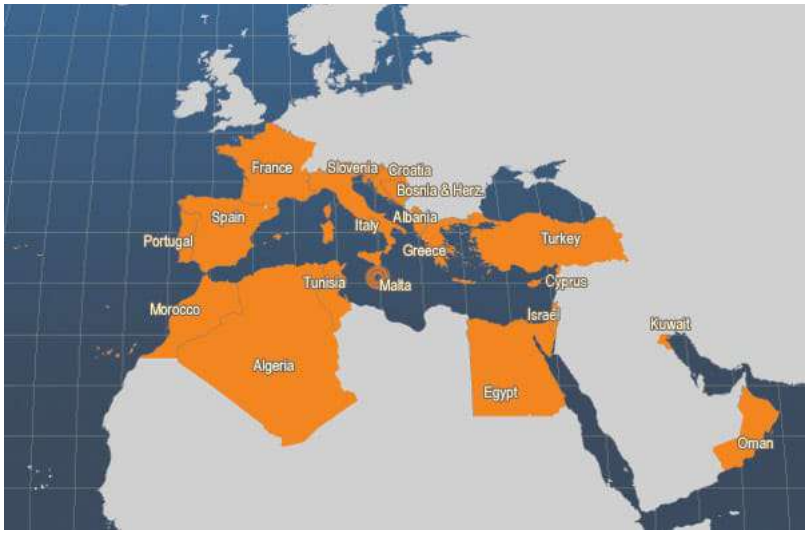
3.2 Seabream

Sparus aurata (Figure 4), commonly known as the gilthead seabream, holds significant ecological importance and is the most frequently cultivated marine fish in the Mediterranean region and southern Europe. Although it has hints of both black and yellow, sea bream's primary color is silver (Figure 5).The Italian "vallicoltura" and the Egyptian "hosha" are examples of large-scale fish-rearing systems that operate as natural fish traps by utilizing the natural trophic flow of juveniles predominantly from the sea into coastal lagoons.

Figure 4: Sea Bream (*Sparus aurata).*

The gilthead seabream is a highly suitable species for large-scale aquaculture in the Mediterranean region(Figure 5) owing to its high survival rate, favorable market price, and dietary preferences. The successful artificial reproduction of juvenile gilthead seabream was achieved in Italy during the period of 1981-1982 and was subsequently accomplished in Spain and Greece in 1988-1989. The successful management of the aquaculture industry includes the hatchery production and farming of this particular fish. As the species is primarily cultivated in captivity, it is susceptible to experiencing inherent fluctuations in response to seasonal variations throughout its maturation phase (Seginer 2016). The aforementioned species exhibited a high degree of adaptability to demanding breeding environments, including both pond and container settings. Its annual yield demonstrated a consistent upward trend until the year 2000, at which point it reached a pinnacle of nearly 87,000 metric tonnes. The global production of this species amounted to approximately 228,000 t in the year 2018. (FAO 2020). The poikilothermic nature of gilthead seabream renders them susceptible to fluctuations in their core temperature, which in turn impacts their food intake, digestion, metabolism, and developmental processes (Jobling 1994).

Figure 5: The Major producer countries of seabream(FAO 2006).



Gilthead seabream was historically farmed in large numbers in saltwater ponds and coastal lagoons, in spite of the emergence of intensive raising methods in the 1980s. Due to its euryhaline and eurythermic nature, the organism is capable of surviving in both freshwater and saltwater environments, as well as tolerating a broad range of water temperatures. In the initial phases of its life cycle, the species can be observed inhabiting marine and brackish water environments, including coastal lagoons and estuaries. Juvenile fish tend to inhabit shallow regions (typically up to a depth of 30 meters), while mature individuals may venture into deeper waters (typically not exceeding a depth of 50 meters). During the spring season, juvenile organisms that are born in the open sea between the months of October and December tend to relocate to coastal waters that are sheltered and offer abundant trophic resources and higher temperatures. In the late autumn season, adult fish engage in reproduction and migrate back to the open sea, despite exhibiting high sensitivity to cold temperatures, with a minimum fatal limit of 4°C. The following table 3 explains the detailed characteristics of Sea Bream. Gilthead seabream is frequently caught in rocky and seagrass (*Posidonia oceanica*) meadows, as well as sandy areas, when found in the open sea. According to Gil A. et el., (2015), the substance in question exhibits a significant concentration of fat-soluble vitamins (A and D), balanced essential amino acids, high-quality proteins, and essential macro- and microminerals (such as iodine, magnesium, phosphorus, and selenium). Marine fish such as Sparus aurata is a viable source of LCPUFA, as noted by Senso et al., in 2017. According to APROMAR's report in 2020, Greece, Turkey, and Spain were ranked as the leading countries in terms of gilthead sea bream production.

Table 3: Characteristics of Sea Bream (*Sparus aurata*) (FAO).

PARAMETER	**CHARACTERISTICS**
Temperature	4-26.5°C.
Habitat	Young fish stay in water no deeper than 30 meters, but adults may go as deep as 50 meters, and they typically catch on sandy coasts.
Diseases in farming	Virus (Lymphocystis, Distended Gut Syndrome (DGS)), Bacteria (Vibriosis, Pasteurellosis (Pseudotuberculosis), Winter Disease Syndrome).
Maturation	20 months for the 350-gram commercial size.
Diet in the wild	Small fish, prawns, crabs, and cuttlefish.
Diet in farming	Artificial feeds (fish meal).
Juvenile phase	45-50 days.
Grow-out	8-16 months.
Distribution in the wild	Mediterranean Sea, Eastern Atlantic coasts from the United Kingdom to Senegal, and the Black Sea
Farming	Spain, Italy, Greece, Turkey, Egypt, Tunisia, and Croatia.
Farming system	Extensive system, semi-intensive system, intensive system, or tank system.

3.3 Sardines

Sardina pilchardus (Figure 6), commonly known as the European sardine, is a diminutive, oily, pelagic fish species that is greatly esteemed for its nutritional properties and industrial uses in the Mediterranean region. The sardine is a diminutive and condensed species, with a length of approximately 4-25 centimeters, and adorned with neatly arranged silver-green scales featuring a lateral blue stripe (Figure 6).The dominant factor attributed to the Croatian purse-seiners, whose annual landings surpass 50,000 tonnes and presently constitute almost 80% of the aggregate catch. The consumption of sardines is significant in terms of diversifying the sources of n-3 fatty acids (FAs). Sardines constitute a significant 70% of fish products, estimated to be around, and are primarily intended for domestic consumption, thus making them one of the principal fishery products.

Figure 6: Sardine (*Sardina pilchardus*).

Furthermore, the minerals found in sardines are predominantly stored in the skeletal structure, albeit they are also detectable in the musculature. Potassium is the most abundant mineral, with a concentration ranging from 300 to 500 mg/kg, which is similar to that found in meat. Sardines are characterized by their elevated levels of phosphorus, which are 8 to 15 times greater than those found in meat, as noted by Lewis-McCrea and Lall (2007) while they contain modest quantities of calcium and, importantly, their sodium content does not exceed that of meat. Sardines are a nutritionally beneficial fish food alternative that is low in calories and carbohydrates, making it a suitable choice for both occasional snacking and regular consumption. Fresh sardines are consumed by individuals during the summer season (June-early October) Zlatanos and Laskaridis (2007). The enhanced fat content, which is believed to enhance their aroma and flavor. the presence of a diverse range of nutrients in sardines. Their meat lack carbohydrates and serve as a notable reservoir of iron, calcium, selenium, magnesium, phosphorous, potassium, fish protein, and vitamins A, B, C, and D. Sardines are a dietary source of coenzyme Q10, which is an antioxidant that has been shown to promote cardiovascular well-being. The advent of novel preservation techniques during the 19th century facilitated the expansion of the canning industry, Santos-Castroviejo (1998). This phenomenon coincided with the growth of the sardine canning industry, Fernández-Casanova in 1998. Towards the end of the 19th century, the canning of sardines had become a significant industry. Nowadays, sardines continue to be a prevalent dietary choice, whether consumed in fresh or canned form, with an average consumption rate of 2.1 kg per individual annually (Martn-Cerdeo, 2010). The table 4 represents the characteristics of Sardine.

Table 4: Characteristics of Sardine (*Sardina pilchardus*) (FAO).

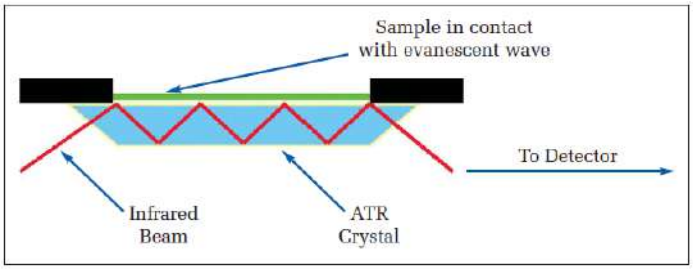
PARAMETER	**CHARACTERISTICS**
Temperature	14-21°C.
Habitat	Deepwater during the daytime (25–55 m); surface during the night (15–35 m).
Diseases	viral hemorrhagic septicemia (VHS)—viral hemorrhagic septicemia virus.
Maturation	1-2 years.
Diet in the wild	Aquatic organisms that float (plankton, zooplancton, copepod crustaceans).
Diet in farming	Artificial feeds (fish meal).
Juvenile phase	20 days.
Grow-out	5 years (max).
Distribution in the wild	Eastern Atlantic Ocean between Iceland and Senegal; the Mediterranean Sea (especially in the Adriatic); and the Black Sea.

4. METHODOLOGY

4.1 ATR-MID IR SPECTROSCOPY

The mid-infrared (MIR) spectroscopy is widely recognized as an accurate and established analytical technique for the acquisition of spectra for solids, liquids, and gases. In the ATR FT-MIR spectroscopy, the specimen is brought into contact with the ATR crystal (Figure 9). In the ATR technique (Figure 7) infrared radiation passes through the crystal and interacts with the specimen located at the surface of the ATR crystal.

Figure 7: Schematic ATR measuring principle

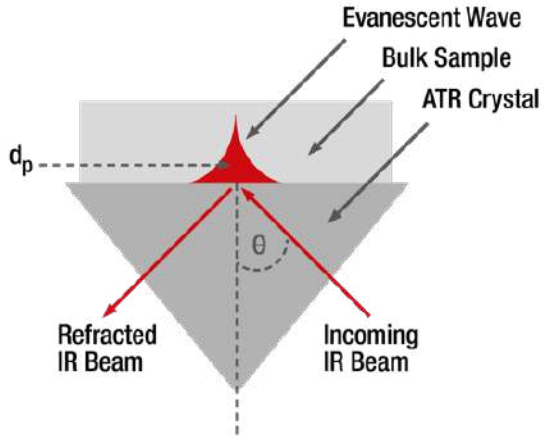


Total internal reflection arises due to the variations in refractive indices between the two materials. The phenomenon is referred to as the emission of a reflection known as the "evanescent wave,"(Figure 8) which has the property of extending into the sample. The absorption of a minute portion of infrared light occurs during the interaction of the evanescent wave with the sample, leading to a total reflection that is slightly attenuated, as inferred from the sample's composition. In total attenuated reflection, the incident beam strikes the sample after having passed through the crystal at an angle of incidence greater than the limit angle and undergoes total reflection; before recrossing the crystal, however, it penetrates the sample and undergoes attenuation on the basis of its characteristic absorptions. The penetration depth is defined as the distance at which the intensity of the radiation a$\frac{1}{e}$ is equal to the initial value and depends on the wavelength of the incident radiation λ and on the refractive indices of the sample and of the IRE according to the relation.

$$d_p = \frac{\lambda}{2\pi \sqrt{n_{IRE}^2 \sin \phi^2 - n_{camp}^2}}$$

λ-Wavelength Of Incident Light In Vacuum
n_1-Refractive Index Of ATR Crystal (Dense Medium)
n_2-Refractive Index Of Sample (Rare Medium)
Θ-Angle Of Incidence

Figure 8: Total internal reflection from (Anton Paar)



There are several ATR attachments available for FTIR spectrometers. They are classified as having a single reflection (one bounce) or multiple reflections (multiple bounce). Different materials are utilized as the ATR crystal depending on the application and the measured samples. Zinc selenide (ZnSe) and diamond are two common materials. To achieve complete internal reflection, ATR crystal(ZnSe) materials must have a refractive index of 2.40. ATR crystals are optically dense due to their high refractive indices and the critical angle of 40°. In spectral regions where the sample absorbs energy, the evanescent wave experiences attenuation within the infrared spectrum. Subsequently, a directed to the detector due to the interferogram structure of the apparatus of the signal received and transferred to an interpersonal computer for mathematically subjected to FT-MIR with the bruker opus 7.0 software, the captured infrared beam and generates an interferogram the processed signal, which can be modified to produce an infrared spectrum.

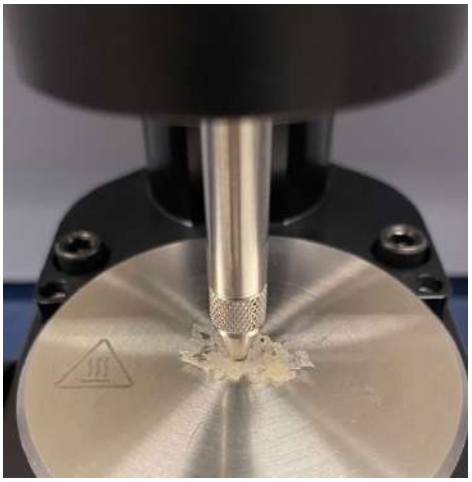


Figure 9: The small piece of the sample is placed on the sample holder of the ATR-mounted mid-IR spectroscopy to facilitate spectral analysis.

4.2 PRINCIPAL COMPONENT ANALYSIS (PCA)

In 1972, Svante Wold and Bruce Kowalski coined the term "chemometrics." Hibbert (2016) posits that chemometrics is a field that involves the application of statistical and mathematical techniques, as well as logical-mathematical methods, in relation to chemistry (Brown et al., 1992). The utilization of this technique is prevalent in numerous fields such as food chemistry, pharmaceutical analysis, agriculture, environmental studies, industrial chemistry, and clinical chemistry (AI Jowder 1999, Holt 1995, and Kim 2005) for the purpose of analyzing vast databases.

The Principal Component Analysis (PCA) method is a commonly utilized approach for reducing a large dataset to a restricted set of independent variables. This technique is effective in preserving and often revealing fundamental trends and patterns within the data. Principal Component Analysis (PCA) is a statistical technique used to analyze a database comprising multiple observations, each of which is characterized by several variables. The first step in PCA involves creating a multidimensional space using all the variables. Within this context, individual observables are denoted by discrete points, and the spatial separation between these points is interpreted as a measure of the similarity between the associated samples. In this context, a linear regression model is utilised to effectively explain the variability of the data points, resulting in the derivation of the initial Principal Component. Subsequently, the residual variance that is yet to be elucidated is attributed to a novel linear amalgamation, thereby generating the second principal component. This process is iteratively repeated, with each successive principal component accounting for variations that are increasingly less noteworthy. The newly created space by personal computers (PCs) is characterised by the "score", which refers to the distance between the projection of an observation on a specific PC and the origin of the space. The spatial arrangement of the scores of various observations in the newly transformed space is indicative of the variability present in the initial dataset.
This transformation has the potential to reveal previously concealed patterns and correlations among the data, which may have been obscured by the sheer volume of information. In order to assess the outcomes of principal component analysis (PCA), two primary instruments are employed. The first is the score plot diagram, also known as the scatter plot of the samples, which displays the location of the representative points of the observables in planes generated by two or three principal components. This plot illustrates the similarities or dissimilarities between observables with respect to the variables that have contributed the most to the specific principal components. The second useful tool that PCA offers is the loading plot graph, which is also known as the dispersion map of variables on major components. It illustrates the impact of each variable on a single PC.

5. Experimental

5.1 The samples details

Three fish species seabass (*Dicentrarchus labrax*), seabream (*Sparus aurata*), and sardines (*Sardina pilchardus*) were chosen because of their importance to the local market and consumer accessibility. The scope of this study encompassed a total of 55 specimens, including wild and farmed, fresh and frozen. In the vicinity of Venice, Italy, samples were gathered from commercial supermarkets (Coop, Consilia, Despar, Conad) and the fish market (*Mercato di Mestre*, Ondablu -*Cannaregiosestiere,* Venice). The table 5 gives detailed information about the samples. The majority of the fresh samples came from the neighborhood fish markets, while the frozen samples were purchased from supermarkets. Whole fish are sold at the farmer's market and need to be prepared prior to whereas fish sold in supermarkets is typically already filleted.

Sea bass (*Dicentrarchus labrax*) is also known as Branzino. A total of 17 fish are evaluated during this process. On average, the fish measured between 35.5 cm^{-1} and 15 cm^{-1} in length. Most of the twelve fish purchased in Venice's supermarkets are exported from Greece and Turkey. The samples that were shipped are supposed to be frozen fish. Three of the fresh fish come from Italy, although the region is not specified. However, two of the fresh fish come from Cavallino-Treporti, which is located close to Venice.

Table 5: Sea Bass (*Dicentrarchus labrax*) Samples information

Provenance	Type	No. of Fish	Seller (Brand)	Type of sample	Average length (cm)
Italy	Fresh/ Farmed	2	Coop	Whole fish	29-32
	Fresh/Semi-Farmed	1	Mestre Market	Whole fish	35
Italy (Cavallino – Treporti)	Fresh/ Semi-Farmed	2	Ondablu	Fillet	29-35.5
Greece	Frozen / Farmed	3	Almaverde Bio	Fillet	18-20
Turkey	Frozen / Farmed	6	Consilia	Fillet	15-23
		3	Despar	Fillet	18-23

Sea Bream *(Sparus aurata)* Orata is the name given to it in Italy, and here we examine 16 distinct sea breams. Eight of the fish investigated are fresh; these come from Ca' di Valle and Veneto, both of which are only partially farmed. The remaining eight fish specimens are frozen and come from farms in Turkey and Greece. The length of the fish, on average, ranged from 14 to 32 cm in length, depending on the type of fish and the vendor. These samples were obtained from Ondablu, MestreMarket, Coop, Despar, and Almaverde Bio. Following informations are mentioned in table 6.

Table 6: Sea Bream *(Sparus aurata)* Samples information

Provenance	**Type**	**No. of Fish**	**Seller (Brand)**	**Type of sample**	**Average length(cm)**
Ca' di Valle	Fresh/ Semi-Farmed	3	Ondablu	Whole fish	28-31.5
Veneto	Fresh/ Semi-Farmed	1	Mestre Market	Whole fish	32
Italy	Fresh/ Farmed	4	coop	Fillet	25-30.3
Turkey	Frozen/ Farmed	2	coop	Whole fish	18-20
	Frozen/ Farmed	3	Despar	Fillet	16-19.5
Greece	Frozen/ Farmed	3	Almaverde Bio	Fillet	14-19

Sardine (*Sardina pilchardus*) (Figure 4) is a popular fish, all the sardines were purchased fresh and to obtain frozen samples we proceeded by freezing at a temperature below 0ºC. In the investigation of sardines, a total of 22 samples were collected, with 12 fresh fish and 10 frozen fish coming from the Veneto region in Italy and being acquired from Mestre Market and Ondablu. In all, there were 22 samples: 11 from the Mestre Market (4 fresh fish and 7 frozen fish samples) and another 11 samples from the Ondablu Market (9 fresh and 3 frozen fish samples).

Table 7: sardines (*Sardina pilchardus*) Samples information.

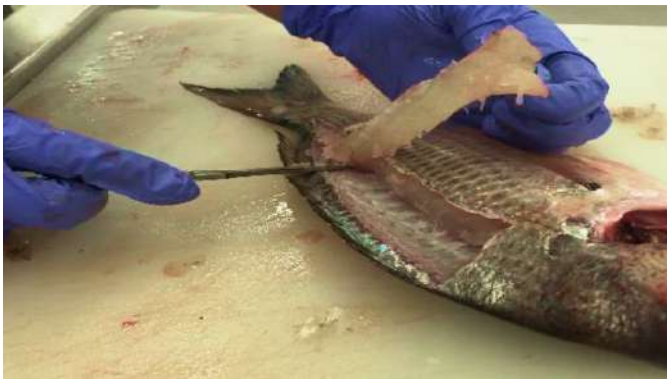
Provenance	**Type**	**No. of Fish**	**Seller (Brand)**	**Type of sample**	**Average length (cm)**
Italy, Veneto (Chioggia)	Frozen/ Wild	1	Ondablu	Whole fish	9
Veneto	Fresh/ Wild	4	Mestre Market		7-15
	Frozen/ Wild	7		Whole fish	4.5-10
Veneto	Fresh	8	Ondablu	Whole fish	10-14.5
Veneto	Frozen	2			10-14.5

5.2 Methods of samples preparation

The processing of the fish sample is a complicated procedure that requires a high level of precision as well as a significant amount of time. Fillets and entire fish were both processed from fish in order to fulfill the requirement of obtaining a sample that is representative. It is important to work with the sample in order to lower the probability of contamination. All of the operations are performed in a clean environment, and the important pieces of equipment have been organized in such a manner that they are easy to reach in order to facilitate the dissections.

Fish dissection: This involves measuring the weight and length as well as removing the skin, scales, and bones. The muscle in the entire fish is speared by using forceps and a surgical blade to remove the skin and the muscle portion without damaging the bones. In order to prepare the fillet, the white flesh must first be taken from the skin, and one must also ensure that the muscle is devoid of bone. Just above the lateral line, it is located at the central part of the fish and between its dorsal and caudal fins, which obtain samples of the myomeres (flesh). This will result in a greater amount of muscle tissue being gathered while also lowering the risk of accidentally piercing any of the internal organs. The ideal position is such that it lies just below the fish's lateral line (Figure 8) to prevent piercing the fish's lower intestine or any of its other internal organs. In the event that the gut is exposed, the whole fish will become infected and unfit for the procedure.

Figure 10: Dissection of the Muscle.



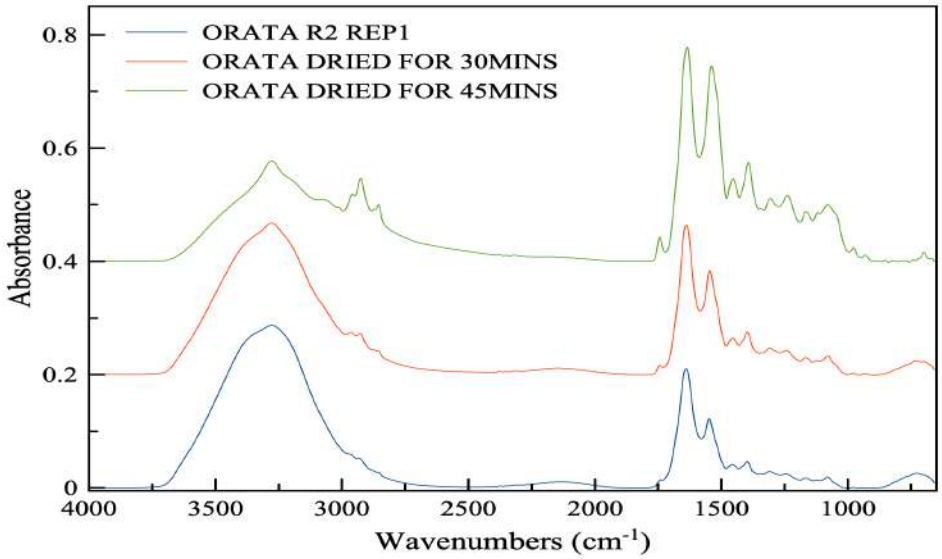
Preparation of samples: In the absence of myosepta (white connective tissue) and red muscle, myomeres (white flesh) are then collected. Prior to the analysis, a homogenized sample is obtained by first carefully blending the myomeres (clean flesh) in a food processor. (Elvingson and Sjaunja, 1992; Okpanachi et al., 2018). The sample paste is obtained without the incorporation of aqueous or other fluid constituents. The minced sample is then sieved through two sets of net gap sieves, with the largest gap measuring 0.420 µm(Figure 11) and the smallest measuring 0.063 µm, to remove any myosepta (white connective tissue) to achieve homogenous samples. This procedure was carried out with all 55 fish, which included 17 seabass, 16 seabream, and 22 sardines.

Figure 11: The muscle sample in the sieve (0.420 μm)

5.3 The role of moisture in the preparation of the samples

The elevated level of moisture present in fish poses a major obstacle in the utilisation of MIR spectroscopy for the purpose of fish tissue analysis. Research has demonstrated that a significant proportion of fish species exhibit a moisture content ranging from 60% to 80%. For example, Figure 12 displays the absorption peaks observed in the spectrum, which are contingent upon the fluctuations in the water content within the sample. The lowermost spectra exhibited spectral windows spanning the ranges of 4000-2500 cm−1 and 1600-950 cm−1, thereby validating the presence of water.

Figure 12: FTIR spectral representation for the 3 samples with different water content

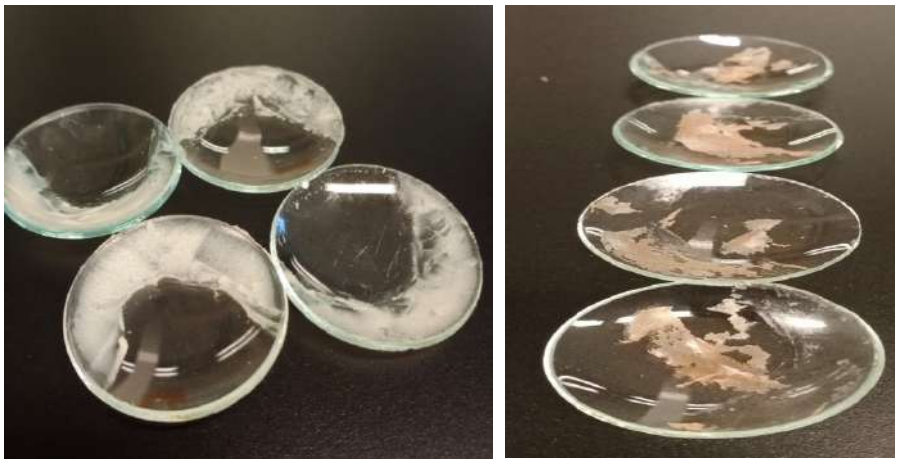


The implication is that the spectral signatures will exhibit contributions from H2O. Upon examination of the second and third spectra from the lowermost portion, which is from the dried fish specimen, it is evident that supplementary peaks are present. These peaks correspond to the presence of protein, fat, and amino acids. The alterations observed in the sample were attributed to the progressive reduction in its moisture content, as reported by J. Murray & Co. (2001), H. H. Huss (1995), and EFSA (2005).In order to comprehend the chemical compositions of various species, The removal of moisture is a crucial step in achieving optimal outcomes. The sample plates are subjected to vacuum drying conditions within an M40-VT oven while maintaining a temperature of 35 degrees Celsius. This is achieved under conditions of consistent oven temperature. Both the fresh and frozen fish samples were subjected to identical treatment protocols, with the sole exception being the duration of exposure. Specifically, the fresh fish samples were subjected to a 35-40 minute exposure period, while the frozen fish samples were exposed for a duration of 40-45 minutes.

5.4 Purpose of Multiple Sampling

For the purposes of this study, each distinct species of fish is separated into four separate duplicates. In order to get accurate data of the spectrum, the sample is grounded to create a homogeneous paste before being processed. This paste has been given a fixed weight of 2.0 grams per sample plate and has been spread uniformly throughout all four testing plates (Figure 13 left). The previously described plates, each of which is covered in an even layer of fish paste, are put through a procedure known as vacuum drying, which requires the application of both a vacuum and a temperature of 35°C for a period of time ranging from 35 to 45 minutes (Figure 13 Right).

Figure 13: The wet sample of processed fish paste was evenly distributed on the sample plate (Left). The sample plate contains dehydrated fish samples (Right).The desiccated specimens are



employed in the analysis of spectra. Each fish sample (as shown in Figure 14) was subjected to replication, resulting in four individual spectra. The act of replicating samples is deemed inadequate for both visualization and measurement objectives, thereby requiring the acquisition of an average spectrum to facilitate subsequent analysis. The utilization of these four spectra is employed to derive the mean spectrum for analytical purposes. This methodology enables the understanding of individual spectra.

Figure 14: The four-replication spectrum of Orate samples

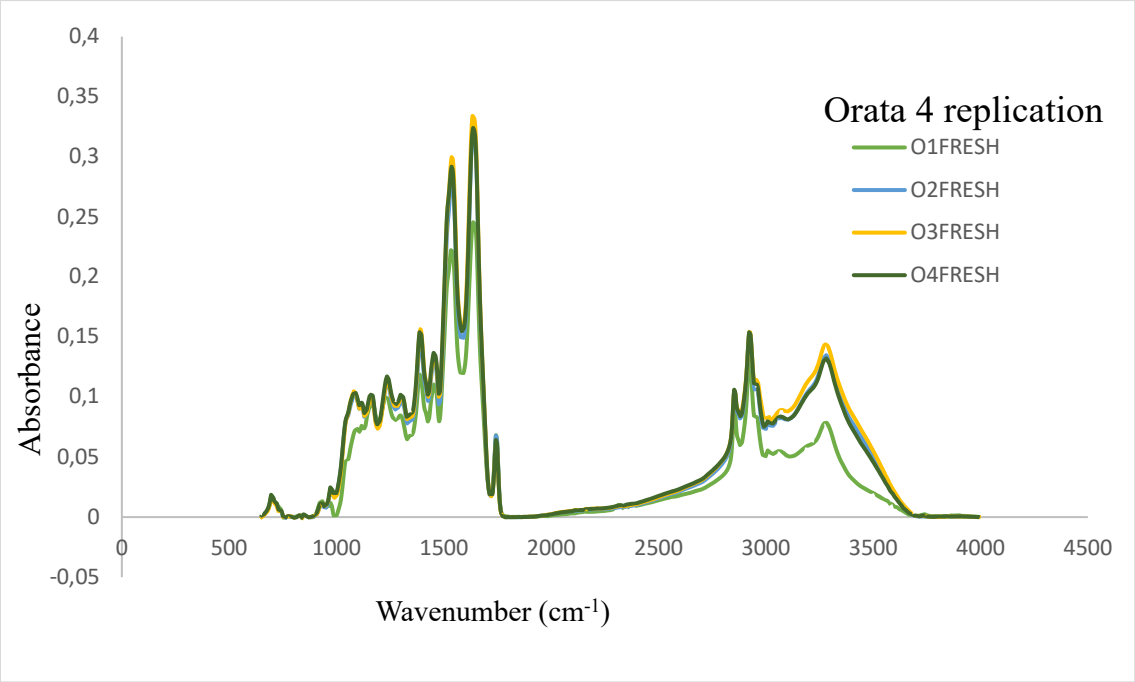
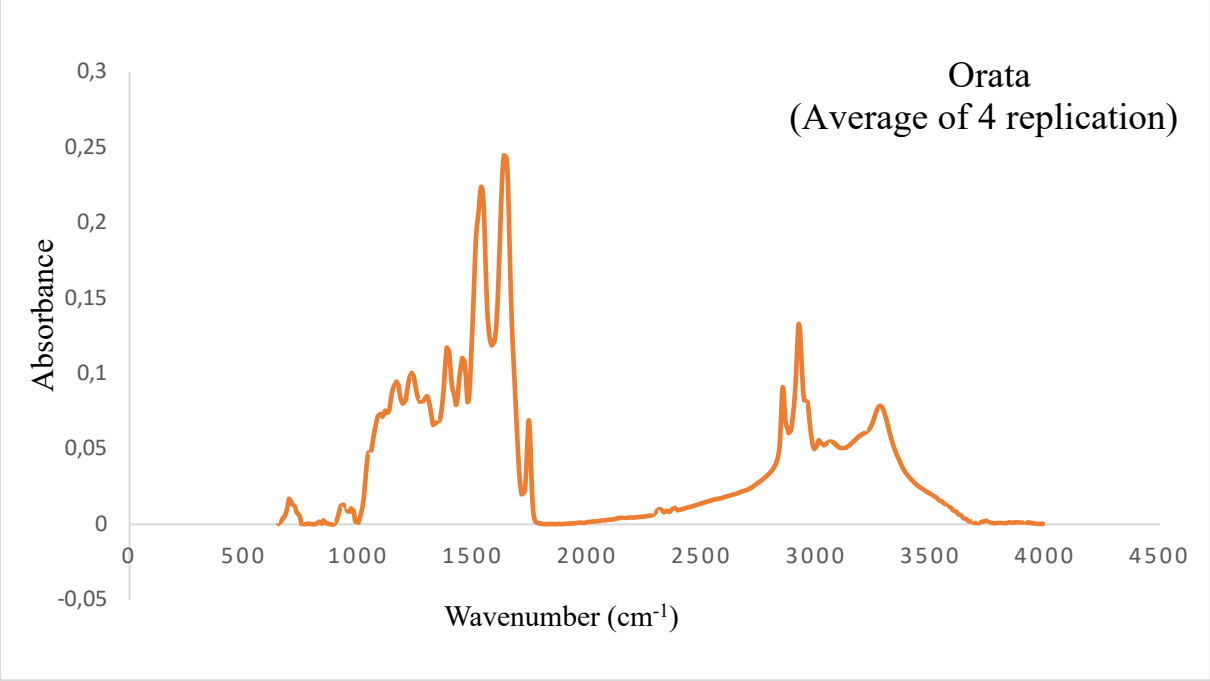


Figure 15: The Average spectra from the four replication spectra of Orate samples



6. SPECTRAL ANALYSIS

6.1 Literature data

The MIR spectra illustrate multiple absorbance bands that result from fundamental transitions. The spectra analysis involves examining two primary regions, namely the functional group region (4000–1300 cm^{-1}) and the fingerprint region (1300–600 cm^{-1}). This analysis enables the determination of a molecular fingerprint. According to (Thompson 2018), the functional group region can be divided into distinct zones that correspond to specific functional groups. These zones include the X-H stretching region (4000–2500 cm^{-1}), where X represents C, N, O, or S, the triple-bond stretching region (2700–1850 cm^{-1}), and the double-bond stretching region (2000–1500cm^{-1}), which encompasses C=C, C=N, and C=O bonds. The spectral range of 1700–1500cm^{-1} corresponds to the amide I and II bands, revealing details regarding the proteins and their relationship with other elements such as water, ions, and other proteins. Additionally, the range of 3000–2800 cm^{-1} corresponds to the C-H modes obtained from the methyl and methylene groups of fatty acids, as reported. The spectral distinctions between the fresh and frozen-thawed fish samples were observed within the 1500–900 cm^{-1} range (Karoui et al., 2007). The fingerprint region from 1500 cm^{-1} to 400 cm^{-1} (Al-Jowder et al. 1999; Karoui et al. 2010) is identified by a set of intricate bending vibrations that exhibit various bands, generally overlapping, that are distinctive to the molecular composition of the specimen (Silverstein, R.M. et al. 2015). Despite its intricacy, the fingerprint region has been utilized for identifying adulteration and verifying the authenticity of diverse food items (Roberts, J. et al. 2018). The spectral bands observed at approximately 1048 cm^{-1} are indicative of the primary alcohol, specifically the C−O stretching mode. Meanwhile, the bands observed at approximately 1127 and 1159 cm^{-1} are attributed to the tertiary alcohol, specifically the C−O stretching mode. The observed band at approximately 1239 cm^{-1} was assigned to the asymmetric stretching of phospholipids' PO_2- and the Amide III band, which involves the stretching of C−H and N−H. The spectral feature at approximately 1080 cm−1 is associated with the symmetric stretching of PO_2-, as well as with the vibrations of C−C and C−O bonds in lipids and proteins, as reported (Hernández-Martínez et al. in 2014). The spectral peaks observed at approximately 1460 cm^{-1} correspond to the scissoring of the $-CH_2$ and $-CH_3$ functional groups, while the peak at approximately 1396 cm^{-1} is attributed to the symmetric stretching of the COO– moiety. The bending (rocking) of =CH− (cis) has been detected at approximately 1419 cm^{-1}, which is reported (Vidal et al., 2013).The stretching vibration of N-H bonds, which is a characteristic of protein amino acids, is typically associated with the broad band of moderate intensity observed at approximately 3288 cm^{-1}, as per previous studies, The band located at approximately 1657 $cm^{-1}$is associated with the vibrational motion of N-H bonds in protein amino groups as well as the stretching vibration of alkenes' double bond (C=C). Additionally, the water bands can interfere with the essential spectral characteristics of other substances that are of interest. According to (Safar et al., 1994), water exhibits noticeable infrared absorption, particularly in three distinct bands. These bands are located at 3360 cm^{-1} (The H–O stretching band), 2130 cm^{-1} (the water association band), and 1640 cm^{-1} (the H–O–H bending vibration). Failure to dry samples may result in the masking of the N-H bond band by the water hydroxyl (O-H) bond vibration. (Meza-Márquez 2010; Hernández-Martnez 2013; Papadopoulou 2011; Carbonaro and Nucara 2010; Wu et al. 2008; Rohman 2011; Shiroma and Rodriguez-Solona 2009). C-H stretching vibrations, both symmetric and asymmetric, are represented by peaks at

about 2925 cm^{-1} and 2854 cm^{-1}.(Guillen 2000; Papadopoulou 2011; Ordoudi et al. 2014). In proteins and fatty acids, these connections are shown by the methylene (CH_2) and methyl (CH_3) groups, respectively. The carbonyl bond stretching vibration, which can be detected as well as esters and free fatty acids, is accountable for the peak in the spectrum that can be observed in the range of 1746 cm^{-1}.The spectral characteristic observed at 1542 cm^{-1} exhibits attributes similar to those of the amides II and is an association of the vibrational modes with N-H bond bending and C-H bond stretching. The wavenumbers are associated with the standard vibration modes of the fatty acid lipids. (Hernández-Martnez 2013; Shiroma and Rodriguez-Solona 2009; Rohman 2011).The follwing table 8 will provide detailed information about the chemical groups associated with wavenumbers.

Table 8: Wavenumbers assignment to the chemical functional groups.

Functional group/assignment	**Wavenumber (cm^{-1})**
1. Saturated Aliphatic (alkene/alkyl) a) Methyl (−CH_3)	
a) Methyl (−CH_3)	
Methyl C-H asym./sym.	2970–2950/2880–2860
Stretch Methyl C-H asym./sym.	1470–1430/1380–1370
Bend gem-Dimethyl or ‘‘iso’’- (doublet)	1385–1380/1370–1365
Trimethyl or ‘‘tert-butyl’’ (multiplet)	1395–1385/1365
b) Methylene (>CH_2)	
Methylene C-H asym./sym. Stretch	2935–2915/2865–2845
Methylene C-H bend	1485–1445
Methylene —$(CH_2)_n$— rocking ($n \geq 3$)	750–720
Cyclohexane ring vibrations	1055–1000/1005–925
c) Methyne(>CH−)	
Methyne C-H stretch	2900–2880
Methyne C-H bend	1350–1330
Skeletal C-C vibrations	1300–700
d) Special methyl (−CH_3) frequencies	
Methoxy, methyl ether O-CH_3,	2850–2815
C-H stretch Methylamino, N-CH_3, C-H stretch	2820–2780

Functional group/assignment	Wavenumber (cm^{-1})
2. Olefinic(alkene)	
Alkenyl C=C stretch	1680–1620
Aryl-substituted C=C	1625
Conjugated C=C	1600
Terminal (vinyl) C-H stretch	3095–3075/3040–3010
Pendant (vinylidene) C-H stretch	3095–3075
Medial, cis- or trans-C-H stretch	3040–3010
Vinyl C-H in-plane bend	1420–1410
Vinylidene C-H in-plane bend	1310–1290
Vinyl C-H out-of-plane bend	995–985 + 915–890
Vinylidene C-H out-of-plane bend	895–885
trans-C-H out-of-plane bend	970–960
cis-C-H out-of-plane bend	700 (broad)
3. Aromatic ring (aryl)	
C=C-C Aromatic ring stretch	1615–1580 / 1510–1450
Aromatic C-H stretch	3130–3070
Aromatic C-H in-plane bend	1225–950 (several)
Aromatic C-H out-of-plane bend	900–670 (several)
C-H Monosubstitution (phenyl)	770–730 + 710–690
C-H 1,2-Disubstitution (ortho)	770–735
C-H 1,3-Disubstitution (meta)	810–750 + 900–860
C-H 1,4-Disubstitution (para)	860–800
Aromatic combination bands	2000–1660 (several)

Functional group/assignment	Wavenumber (cm^{-1})
4.Acetylenic(alkyne)	
C≡C Terminal alkyne (monosubstituted)	2140–2100
C≡C Medial alkyne (disubstituted)	2260–2190
Alkyne C-H stretch	3320–3310
Alkyne C-H bend	680–610
Alkyne C-H bend	630 (typical)
5. Aliphatic organohalogen compound	
Aliphatic fluoro compounds, C-F stretch	1150–1000
Aliphatic chloro compounds, C-Cl stretch	800–700
Aliphatic Bromo compounds, C-Br stretch	700–600
Aliphatic iodo compounds, C-I stretch	600–500
6. Alcohol and hydroxy compound	
Hydroxy group, H-bonded OH stretch.	3570–3200 (broad)
Normal ‘‘polymeric’’ OH stretch	3400–3200
Dimeric OH stretch.	3550–3450
Internally bonded OH stretch	3570–3540
Nonbonded hydroxy group, OH stretch	3645–3600 (narrow)
Primary alcohol, OH stretch	3645–3630
Secondary alcohol, OH stretch	3635–3620
Tertiary alcohol, OH stretch	3620–3540
Phenols, OH stretch	3640–3530
Primary or secondary, OH in-plane bend	1350–1260
Phenol or tertiary alcohol, OH bend	1410–1310
Alcohol, OH out-of-plane bend	720–590
Primary alcohol, C-O stretch	~1050
Secondary alcohol, C-O stretch	~1100
Tertiary alcohol, C-O stretch	~1150
Phenol, C-O stretch	~1200

Functional group/assignment	Wavenumber (cm^{-1})
7. Ether and oxy compound	
Methoxy, C-H stretch (CH_3-O-)	2820–2810
Alkyl-substituted ether, C-O stretch	1150–1050
Cyclic ethers, large rings, C-O stretch	1140–1070
Aromatic ethers, aryl -O stretch	1270–1230
Epoxy and oxirane rings	~1250 + 890–800
Peroxides, C-O-O- stretch	890–820
a) Primary amino	
Aliphatic primary amine, NH stretch.	3400–3380 + 3345–3325
Aromatic primary amine, NH stretch	3510–3460 + 3415–3380
Primary amine, NH bend	1650–1590
Primary amine, CN stretch	1090–1020
b) Secondary amino	
Aliphatic secondary amine, >N-H stretch	3360–3310
Aromatic secondary amine, >N-H stretch	~3450
Heterocyclic amine, >N-H stretch	3490–3430
Imino compounds, =N-H stretch	3350–3320
Secondary amine, >N-H bend	1650–1550
Secondary amine, CN stretch	1190–1130
c) Tertiary amino	
Tertiary amine, CN stretch	1210–1150
d) Aromatic amino	
Aromatic primary amine, CN stretch	1340–1250
Aromatic secondary amine, CN stretch	1350–1280
Aromatic tertiary amine, CN stretch	1360–1310

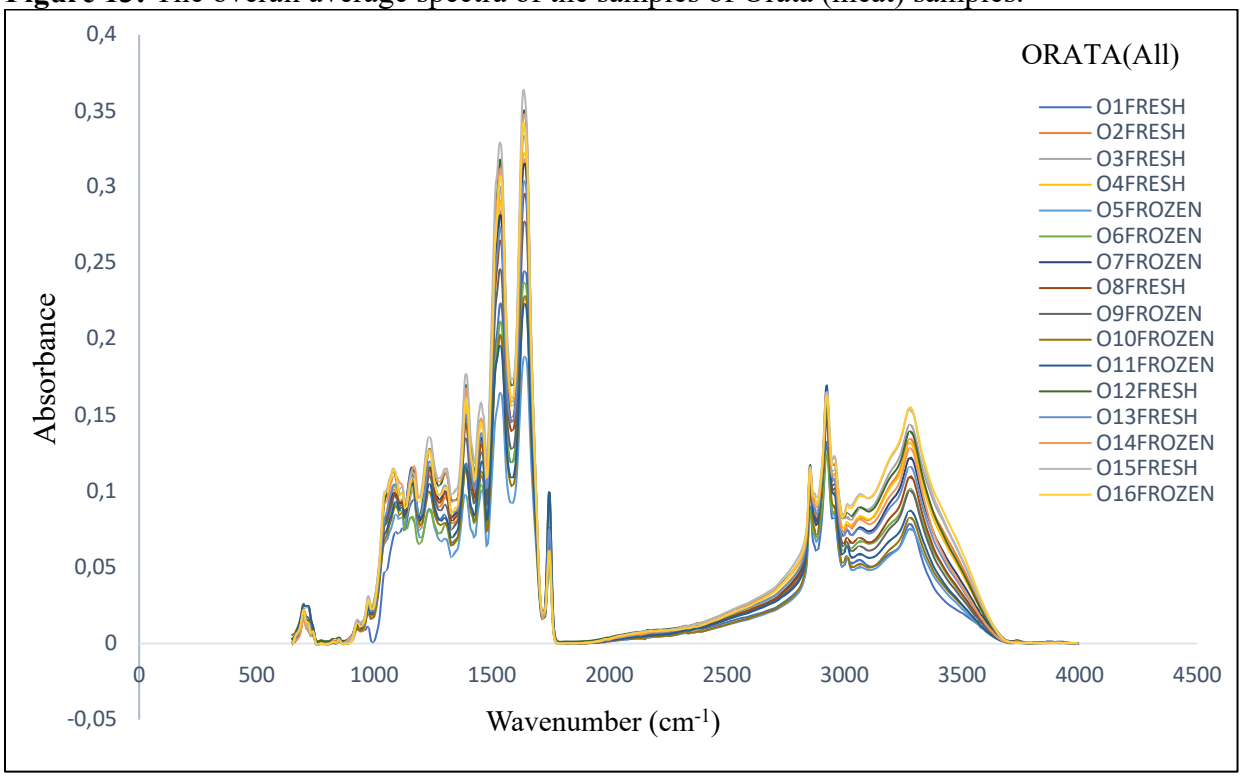
Functional group/assignment	Wavenumber (cm^{-1})
8.Carbonyl compound	
Carboxylate (carboxylic acid salt)	1610–1550/1420–1300
Amide	1680–1630
Quinone or conjugated ketone	1690–1675/(1650–1600)
Carboxylic acid	1725–1700
Ketone	1725–1705
Aldehyde	1740–1725/(2800–2700)
Ester	1750–1725
Six-membered ring lactone	1735
Alkyl carbonate	1760–1740
Acid (acyl) halide	1815–1770
Aryl carbonate	1820–1775
Five-membered ring anhydride	1870–1820/1800–1775
Transition metal carbonyls	2100–1800
9.Nitrogen multiple and cumulated double bond compound	
Aliphatic cyanide/nitrile	2280–2240
Aromatic cyanide/nitrile	2240–2220
Cyanate (-OCN and C-OCN stretch)	2260–2240/1190–1080
Isocyanate (-N=C=O asym. stretch)	2276–2240
Thiocyanate (-SCN)	2175–2140
Isothiocyanate (-NCS)	2150–1990
Open-chain imino (-C=N-)	1690–1590
Open-chain azo (-N=N-)	1630–1575
10.Common inorganic ions	
Carbonate ion	1490–1410/880–860
Sulfate ion	1130–1080/680–610
Nitrate ion	1380–1350/840–815
Phosphate ion	1100–1000
Ammonium ion	3300–3030/1430–1390
Cyanide ion, thiocyanate ion, and related ions	2200–2000
Silicate ion	1100–900

11.Simple hetero-oxy compounds	
a) Nitrogen-oxy compounds	
Aliphatic nitro compounds	1560–1540/1380–1350
Organic nitrates	1640–1620/1285–1270
Aromatic nitro compounds	1555–1485/1355–1320
b) Phosphorus-oxy compounds	
Organic phosphates (P=O stretch)	1350–1250
Aliphatic phosphates (P-O-C stretch)	1050–990
Aromatic phosphates (P-O-C stretch)	1240–1190/995–850
c) Sulfur-oxy compounds	
Dialkyl/aryl sulfones	1335–1300/1170–1135
Organic sulfates	1420–1370/1200–1180
Sulfonates	1365–1340/1200–1100
d) Silicon-oxy compounds	
Organic siloxane or silicone (Si-O-Si)	1095–1075/1055–1020
Organic siloxane or silicone (Si-O-C)	1110–1080
13.Thiols and thio-substituted compounds	
Thiols (S-H stretch)	2600–2550
Thiol or thioether, CH2-S-(C-S stretch)	710–685
Thioethers, CH_3-S-(C-S stretch)	660–630
Aryl thioethers, ø-S (C-S stretch)	715–670
Disulfides (C-S stretch)	705–570
Disulfides (S-S stretch)	620–600
Aryl disulfides (S-S stretch)	500–430
Polysulfides (S-S stretch)	500–470

6.2 Spectral Analysis

The mid-infrared spectra of Orata, Branzino, and sardine meat in both fresh and frozen states have been depicted in Figure 13(Orata), Figure 14(Branzino), and Figure 15(Sardine), respectively. In regards to the visual arrangement, the spectral features of each distinct species display a similar appearance. Multiple spectral regions were utilized in the analysis, specifically, the ranges of 2900-2827 cm^{-1} and 1782-1705 cm^{-1}, which correspond to the fat content, 1701-1507 $cm^{-1}$for protein content, and 1200-967 (insert the spectra of for all the fish in each and every interval) cm^{-1} for carbohydrates. The spectral analysis revealed the presence of a significant band within the range of 1700 to 1600 cm^{-1}, which can be attributed to the amide I band of the proteins and the O–H bond contribution.

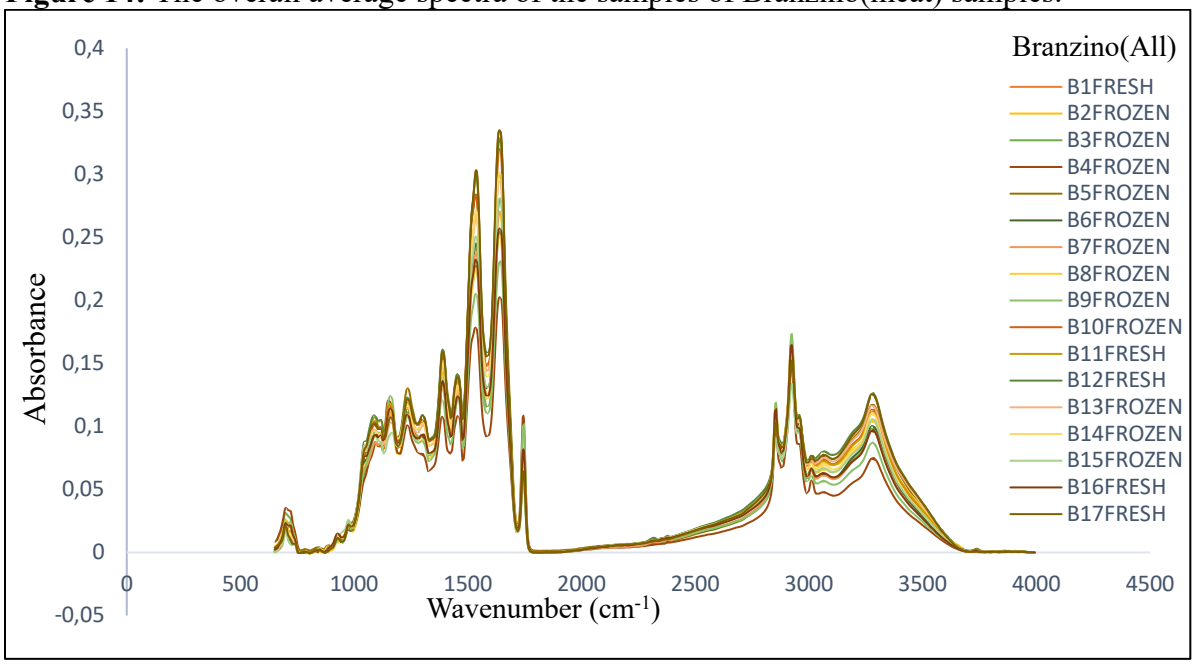
Figure 13: The overall average spectra of the samples of Orata (meat) samples.



The O-H stretching of amide I is the prominent characteristic observed at approximately 1640 cm^{-1} (Figure 13), 1639 cm^{-1} (Figure 14), and 1630 cm^{-1} (Figure 15), with a significant contribution from protein. The amide II absorption of protein is represented by the second-largest peaks, which are observed at 1537 cm^{-1} (Figure 13), 1535 cm^{-1} (Figure 14), and 1530 cm^{-1} (Figure 15). An additional band was detected at a wavenumber of 1550 cm^{-1}. The maximum point detected at wavelengths of 1744 cm^{-1} (as depicted in Figure 13) and 1743 cm^{-1} (as illustrated in Figures 14 and 15), which can be attributed to the existence of adipose tissue. This phenomenon corresponds to the elongation oscillation of triglycerides towards the ester carbonyl (C=O ester). The present peak exhibits alterations in certain bands in relation to the duration of storage conditions. These spectra show a rise in the C=O peak (1743 cm^{-1}), which may be ascribed to the emergence of ketones and aldehydes, lipid oxidation byproducts. The observed reduction in the intensity of the

amide I band at 1640 cm^{-1} was attributed (can be) to protein rupture, an increase of the band's intensity at 1168 cm^{-1}, which corresponds to the stretching vibration of the C–O ester groups. The spectra indicate the presence of minor intensity peaks at various wavenumbers, including 1448, 1455, and 1456 cm^{-1}, which to the scissoring of fat, $–CH_2$, and $–CH_3$ groups.

Figure 14: The overall average spectra of the samples of Branzino(meat) samples.



Additionally, peaks were observed at 1384-1389 cm^{-1}, which correspond to the symmetric stretching of the COO– group, and at 1233–1237 cm^{-1}, which correspond to the asymmetric stretching of phospholipids and amide III bands (C–H and N–H). The peaks observed at 1163–1155 cm^{-1} correspond to the C–O–C asymmetric stretching of lipid esters, while the peak at 1160 cm^{-1} corresponds to $–CH_2$ bending and C–O stretching vibrations. Finally, a peak was observed at 1084 cm^{-1}, which corresponds to the symmetric stretching of PO_2 and C–C and C–O of lipids and proteins. The observed peaks at 2958, 2996, 2945, 2923, 2925, and 2854 $cm^{-1}$are attributed to the asymmetric stretching of methyl ($–CH_3$), asymmetric stretching of methylene ($–CH_2$), and symmetric stretching of methylene (CH_2), respectively. The MIR spectra exhibit a wide band about 3600 to 3000 cm^{-1}, which can be attributed to the vibrational motion of the O-H bond in water (3360 cm^{-1}) and the amide II group present in proteins (3270 cm^{-1}). all the above considered variation in the noted different spectra. The variation between the average spectrum of Fresh and Frozen samples of Orata, Branzino and Sardines is shown in the Figure 16-18. This shows subtle difference and is hard to understand through the spectral data. To make this analysis more efficient PCA Principal Component Analysis is Mainly involved.

Figure 15: Average Mid-IR Spectra of sardine (meat) samples.

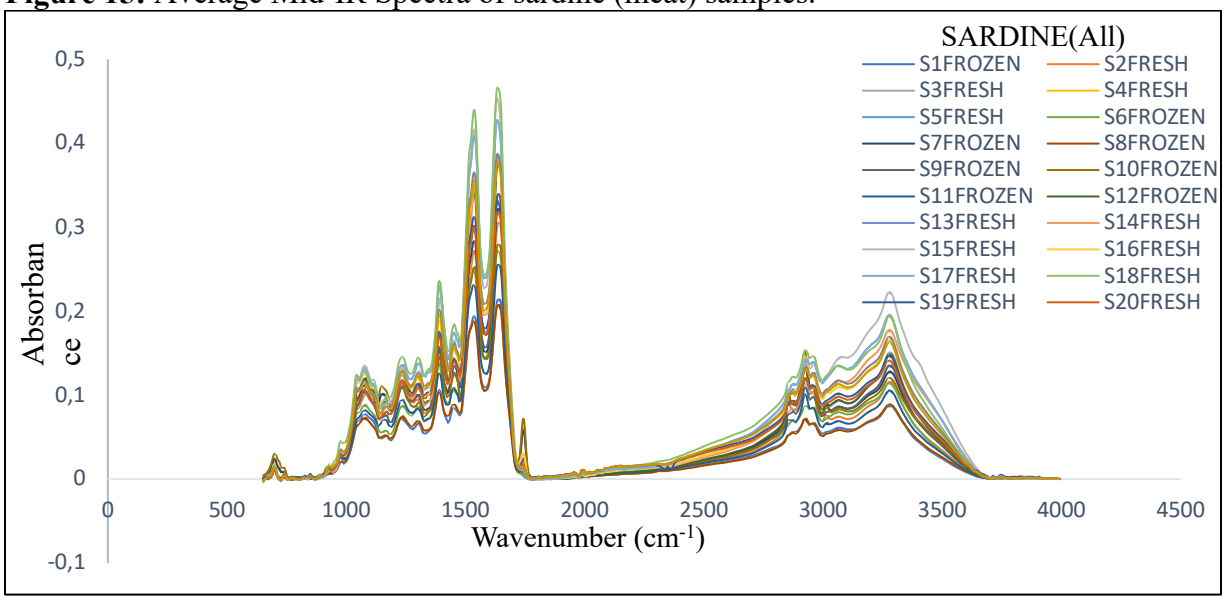


Figure 16: The Spectrum Comparison Between Fresh and Frozen Samples of Orata (Meat)

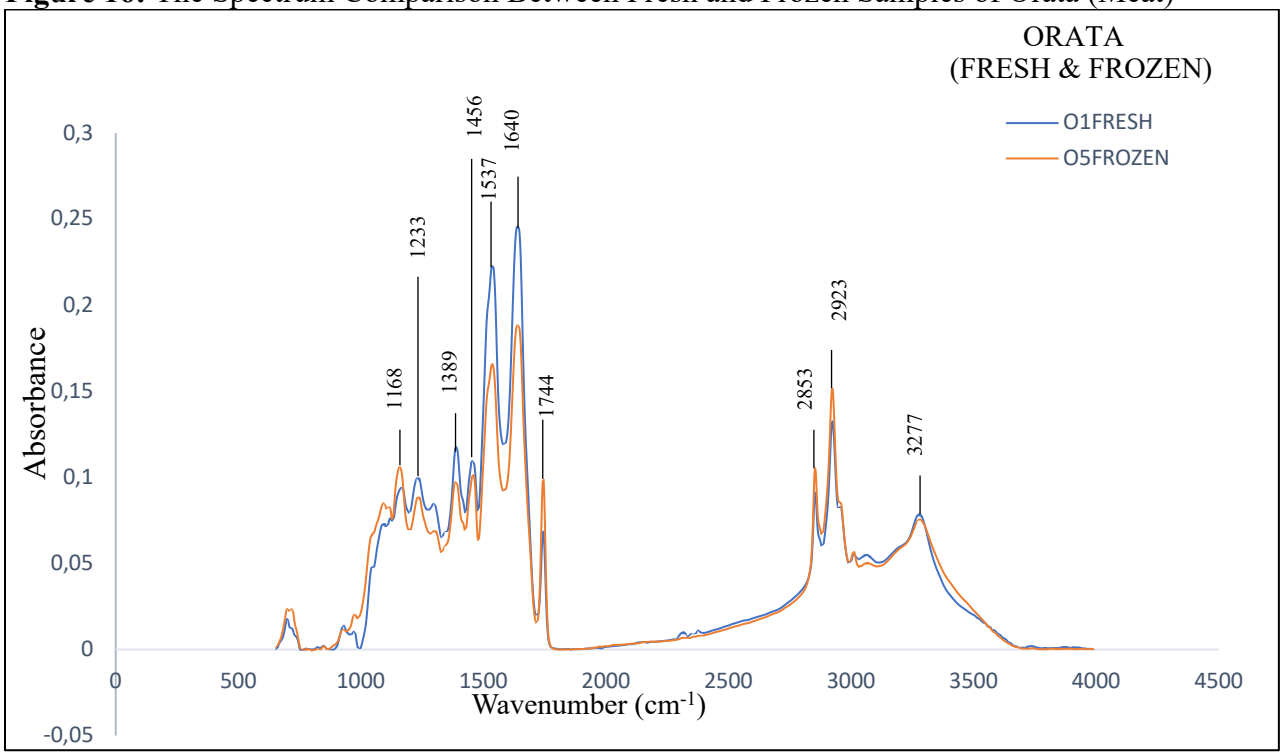


Figure 17: The Spectrum Comparison Between Fresh and Frozen Samples of Branzino (Meat)

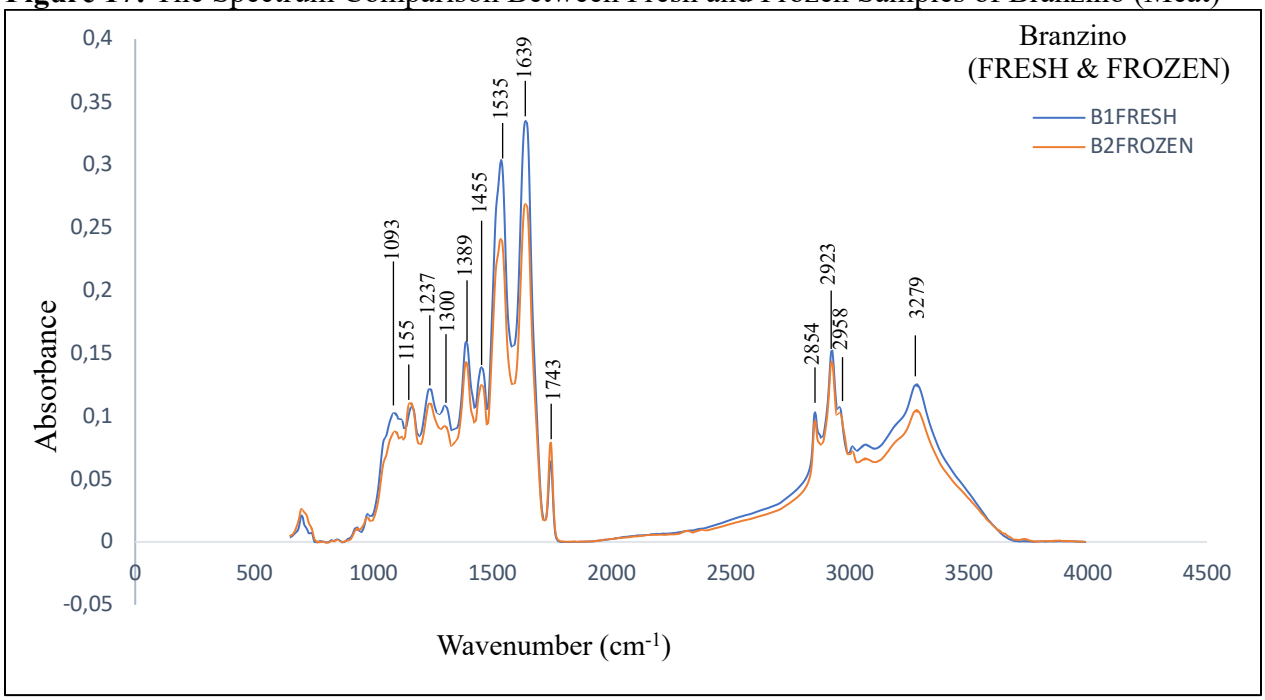
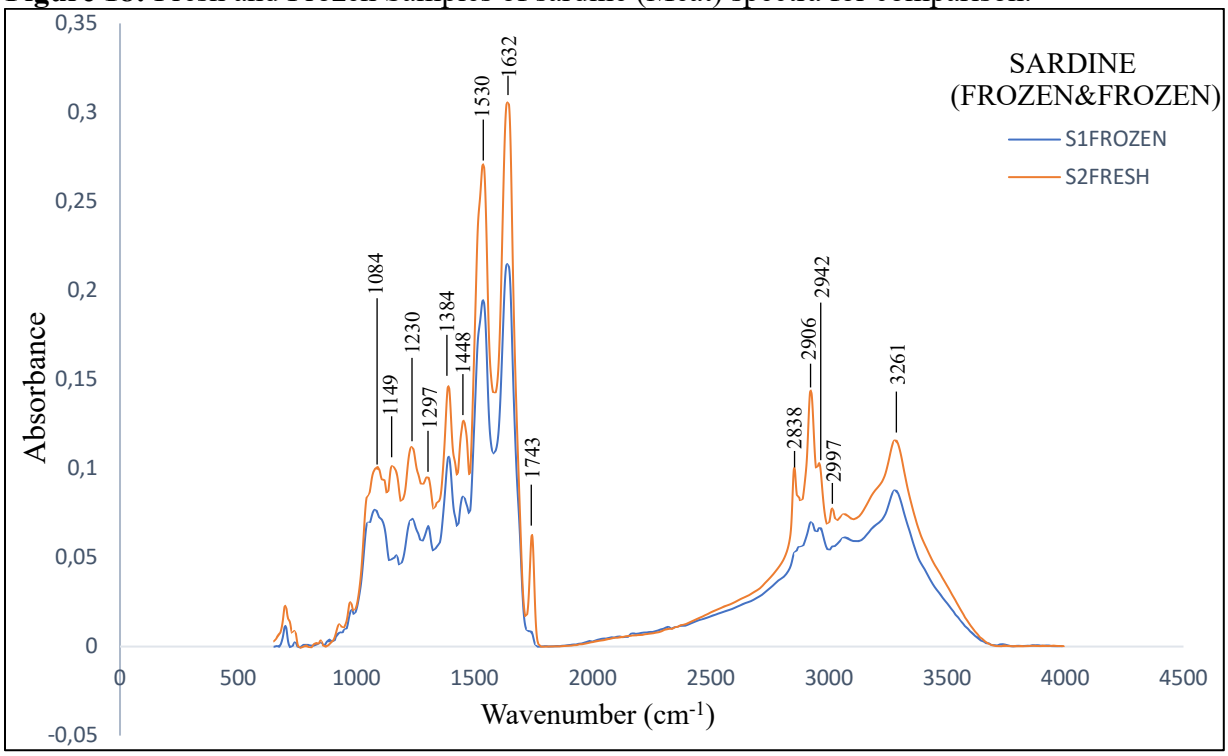


Figure 18: Fresh and Frozen Samples of sardine (Meat) spectra for comparison.



7. PRINCIPAL COMPONENT ANALYSIS

7.1 Discussion outline

The chemometric analysis was applied to the spectroscopic data within specific spectral ranges to investigate the potential for distinguishing the fat and protein content in the meat of three different fish species. Additionally, the study aimed to identify any variations in the samples based on their origin (wild or farmed) and the effects of freezing storage. Throughout the analyses, the data were subjected to fitting via the initial five principal components, and the resultant score plot was assessed. The parameters characterizing the fit quality, specifically the variance accounted for by each principal component, as well as the cumulative R^2 and Q^2 values, representing the explained and predicted portions of the sum of squares, were reported in all instances. The presented data displays solely the score plots that hold significance, accompanied by a brief analysis derived from the loading plots of the corresponding principal components. This approach is utilized to pinpoint the pertinent spectral characteristics.

The present study examined the dissimilarities and similarities in the data while considering the potential grouping of the sample set. Consequently, the spectra, which included either multiple spectra for each sample or averaged ones, were scrutinized for possible differentiation with respect to:

a. The many distinct species (independent of where they originated or how they have been managed for conservation).
b. The conservation treatment (b1 fresh, b2 frozen) is independent of the fish type.
c. The distinction between seabass and seabream flesh.
d. Each fish species' conservation treatment (d1 seabass, d2 seabream, and d3 sardines).
e. The origin of each fish species (e1 seabass, e2 seabream; sardines were excluded since they all came from the Venetian region).

7.2.1 650-1000 cm^{-1} spectral range

In this spectral region, which contains the intense band, omega-3 contributes at a wavelength of around 700 cm^{-1} correspondingly. According to the results of the principal component analysis (PCA).

7.2.1.1 Analysis of all the Spectra

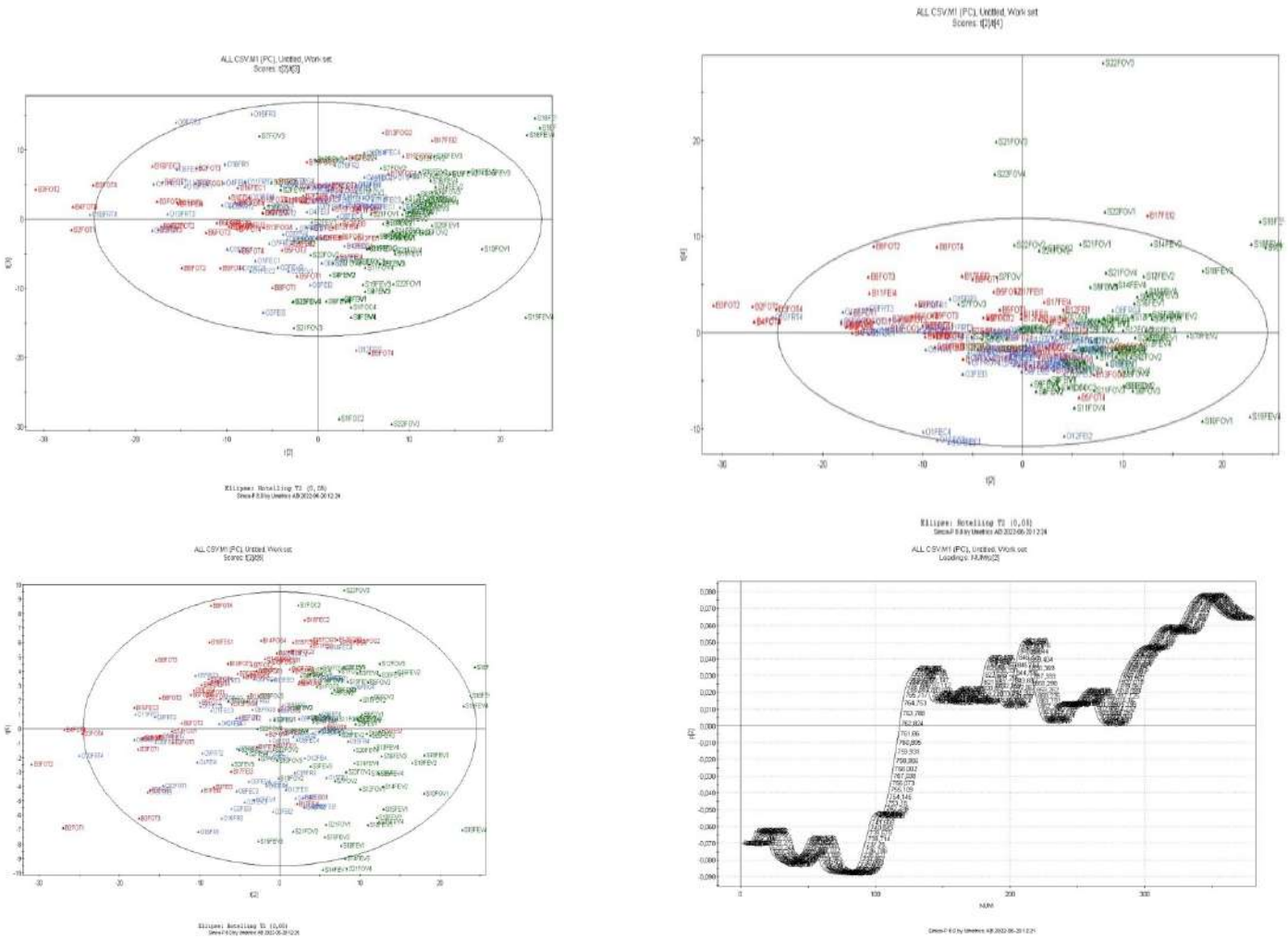
7.2.1.1.a All species

In the table, 9 Shows the fit parameters of the principal component analysis conducted on the dataset that encompasses all the spectra. The dataset comprises 364 variables for 219 observations. The initial five principal components (PCs) explain 99% of the overall variance in the data. The high level of accuracy in the model is demonstrated by the proximity of the Q^2 values to the R^2 values**.**

Table 9: Fit parameters of all the fish data in the 650-1000 cm^{-1} spectral range.

Principal Component	explained variance	cumulative R^2	cumulative Q^2
1	0.382	0.382	0.369
2	0.272	0.654	0.644
3	0.129	0.783	0.772
4	0.063	0.847	0.835
5	0.041	0.888	0.878

Figure 19: PC2 vs PC3(Left), PC4 vs PC2(Right) and PC5 vs PC2(Down Left) scores plots and also PC2(down Right) loading plots of all the samples data in the 650-1000 cm^{-1} spectral range. (seabass = blue, seabream = red, sardines = Green)



The component analysis of PC3 vs PC2, PC4 vs PC2, and PC5 vs PC2 shows that the maximum components are occupied the right side of the planes. The loading plot of PC2, as shown in Figure 28, indicates that the absorptions in the range of approximately 650-1000 cm^{-1} can be correlated with positive values. Therefore, the samples are considered to be representative of omega-3 fatty acids.

7.2.1.1.b1 Fresh

In Table 10 the fit parameters of the PCA performed on the dataset comprising the spectra of all the frozen samples are collected (365 variables for 100 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, that are always close to the R^2 ones.

Table 10: Fit parameters of all the Fresh sample data in the 650-1000 cm^{-1} spectral range

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.432	0.432	0.386
2	0.246	0.678	0.646
3	0.138	0.816	0.794
4	0.054	0.870	0.852
5	0.048	0.918	0.905

Figure 20: PC2 vs PC3(Left), PC2 vs PC4(Right) scores plots of all the fresh samples data in the 650-1000 cm^{-1} spectral range (seabass = blue, seabream = red, sardines = Green).

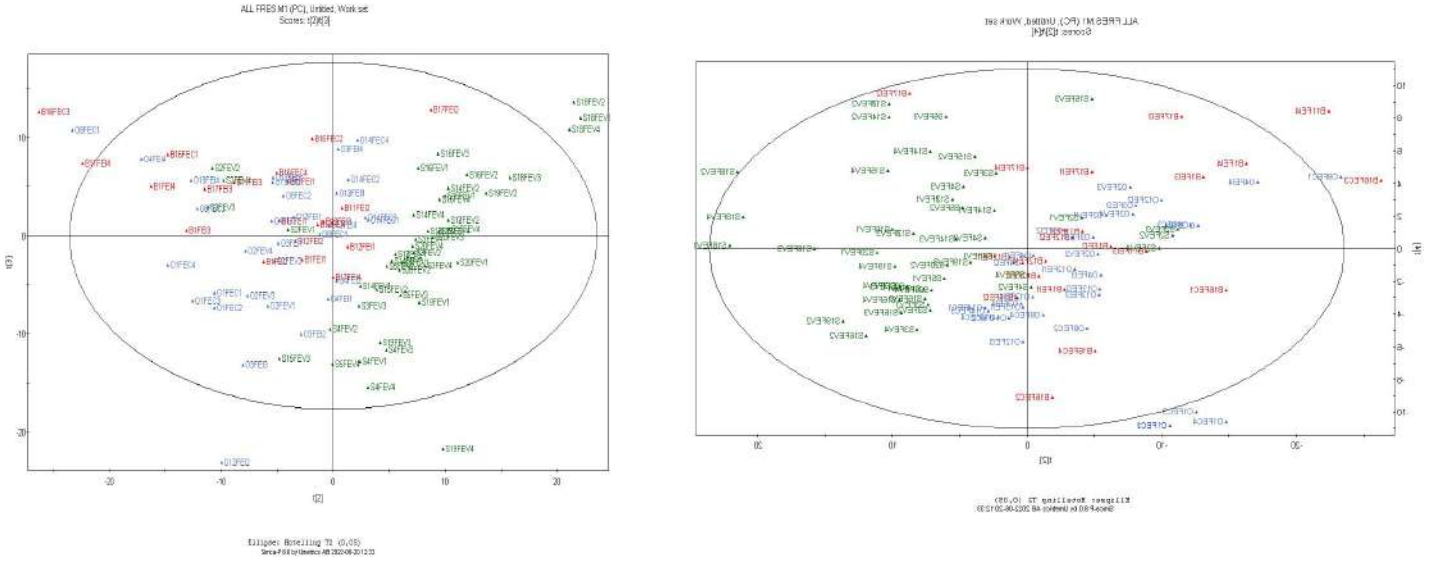
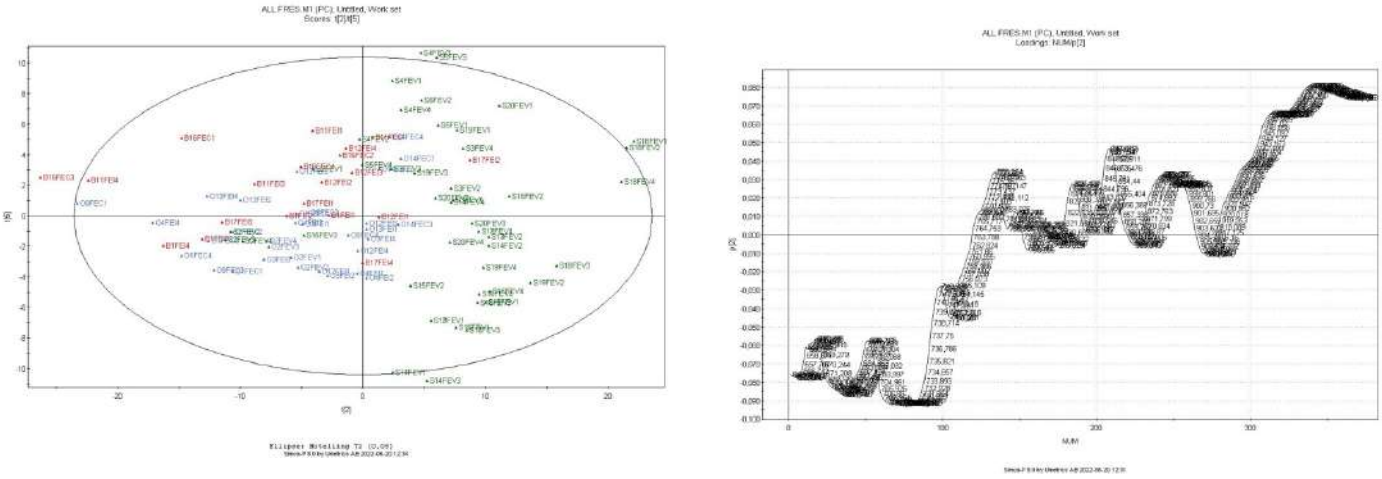


Figure 21: PC2 vs PC5 (Left) scores plot and PC2 (Right) loading plots of all the fresh samples data in the 650-1000 cm^{-1} spectral range.



Based on the observation the components occupied the right side of the plane The loading plot of PC2, as shown in Figure 21(Right), indicates that the absorptions in the range of approximately 650-1000 cm^{-1} can be correlated with positive values. Therefore, the samples are considered to be representative of omega-3 fatty acids.

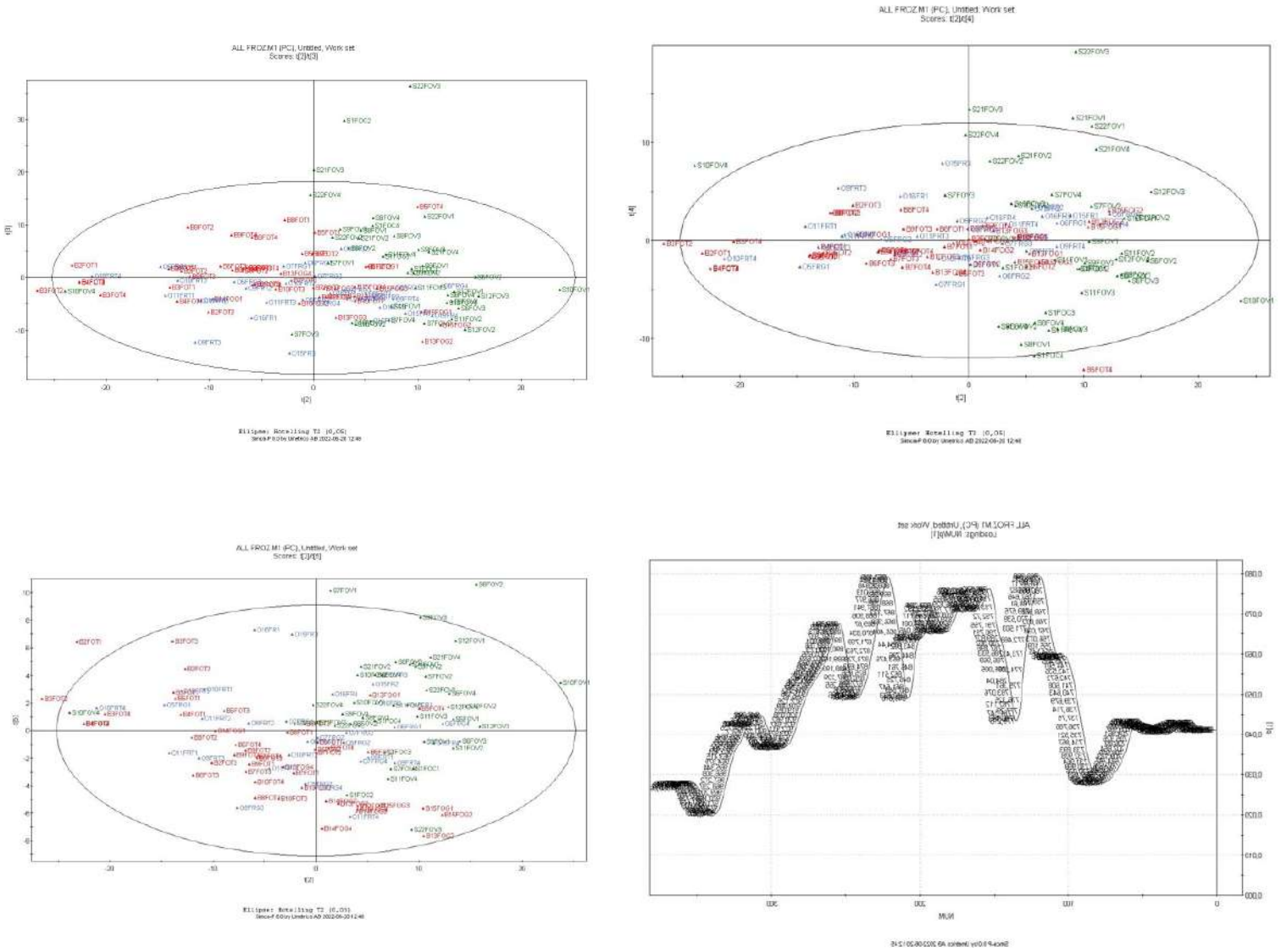
7.2.1.1.b2 frozen

In Table 11 the fit parameters of the PCA performed on the dataset comprising the spectra of all the frozen samples are collected (364 variables for 120 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table11: Fit parameters of all the data in the 650-1000 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.365	0.365	0.312
2	0.284	0.649	0.616
3	0.149	0.799	0.770
4	0.064	0.863	0.839
5	0.037	0.900	0.879

Figure 22: PC2 vs PC3, PC2 vs PC4 and 29: PC2 vs PC5 scores plots and also PC1 loading plots of all the frozen samples data in the 650-1000 cm^{-1} spectral range (seabass = blue, seabream = red, sardines = Green).



The scores plot remains the same as the above-mentioned plots. The loading plot of PC1, as shown in Figure 36, indicates that the absorptions in the range of approximately 650-1000 cm^{-1} can be correlated with positive values. Therefore, the samples are considered to be representative of omega-3 fatty acids.

7.2.1.1.c) seabass vs seabream

In Table 12 the fit parameters of the PCA performed on the dataset comprising the spectra of all the seabass vs seabream samples are collected (364 variables for 132 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, that are always close to the R^2 ones.

Table 12: Fit parameters of all the orate and branzino data in the 650-1000 cm^{-1} spectral range

Principal Component	explained variance	cumulative R^2	cumulative Q^2
1	0.355	0.355	0.345
2	0.202	0.557	0.303
3	0.106	0.663	0.222
4	0.092	0.755	0.260
5	0.036	0.818	0.245

Figure 23: PC2 vs PC1(Left) and PC3 vs PC1(Right) scores plots also of all the samples data of orate and branzino in the 650-1000 cm^{-1} spectral range (seabass = blue, seabream = red).

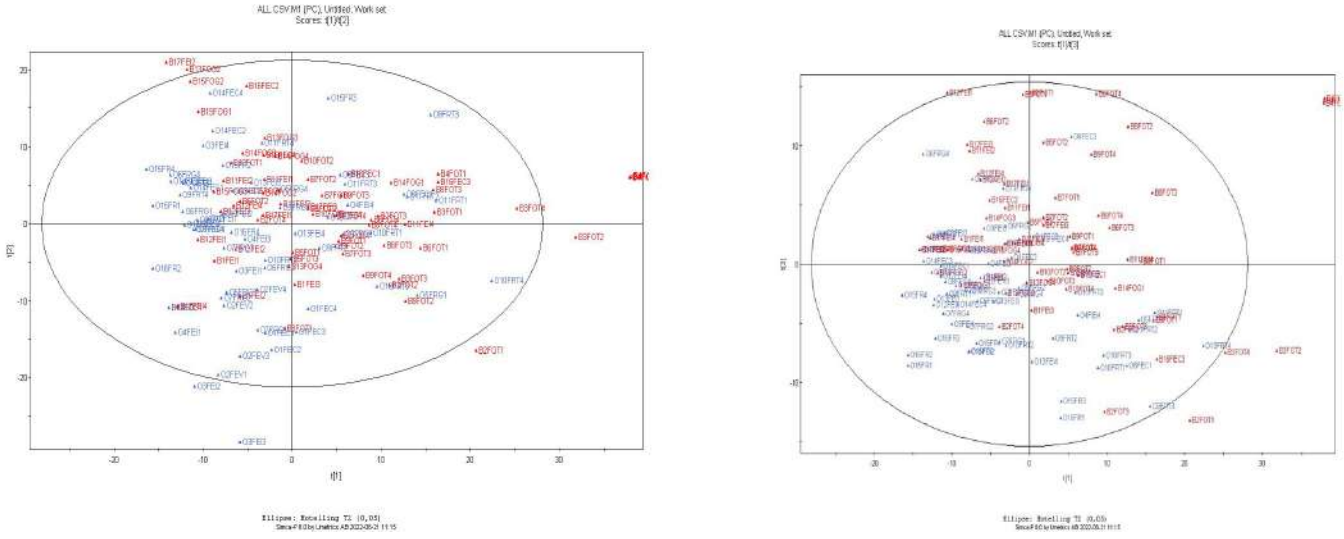
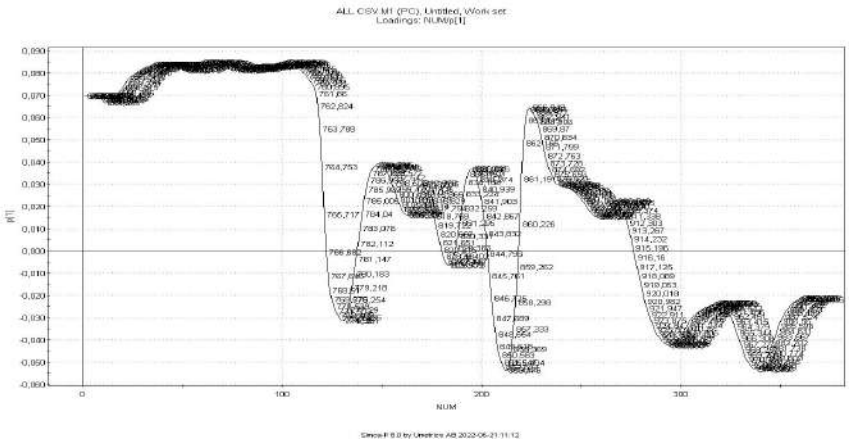


Figure 24: PC1 loading plots of all the Orata and Branzino sample datas in the 650-1000 cm^{-1} spectral range.



The omega-3 features point to the half of the plane in X- axsis.The loading plot of PC1, as shown in Figure 24, indicates that the absorptions in the range of approximately 650-1000 cm^{-1}.

7.2.1.1.d1 Seabass Conservation

In Table 13 the fit parameters of the PCA performed on the dataset comprising the spectra of Seabass data of conservation (Variation Between Fresh and Frozen) of Branzino are collected (364 variables for 68 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components are not displaying any specific Trent based on Conservation.

Table13: Fit parameters of all the sample data of the conservation of Branzino in the 650-1000 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.398	0.398	0.000
2	0.325	0.723	0.520
3	0.104	0.827	0.690
4	0.053	0.880	0.775
5	0.041	0.921	0.846

7.2.1.1.d2 Seabream conservation

In table 14 the fit parameters of the PCA performed on the dataset comprising the data of all the samples based on the conservation of Orata (Fresh and Frozen) are collected (364 variables for 64 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components don't show any particular Trent-based conservation information.

Table 14: Fit parameters of all the sample data of the conservation of Orata at the 650-1000 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.431	0.431	0.003
2	0.236	0.667	0.393
3	0.173	0.841	0.701
4	0.058	0.899	0.806
5	0.040	0.939	0.878

7.2.1.1.d3 Sardines Conservation

In table 15 the fit parameters of the PCA performed on the dataset comprising the spectra of all the samples data based on the conservation of Sardine (Fresh and Frozen) are collected (364 variables for 88 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components don't show any particular Trent-based conservation information.

Table 15: Fit parameters of all the data of the conservation of Sardine at the 650-1000 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.437	0.437	0.404
2	0.194	0.632	0.582
3	0.142	0.774	0.731
4	0.094	0.868	0.837
5	0.043	0.911	0.888

7.2.1.1.e1 Seabass Provenance

In table 16 the fit parameters of the PCA performed on the dataset comprising the spectra of all the samples data based on the provenance of Branzino are collected (348 variables for 68 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components don't show any particular Trent-based conservation information.

Table 16: Fit parameters of all the data about the provenance of Orata in the 650-1000 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.398	0.398	0.000
2	0.325	0.723	0.520
3	0.104	0.827	0.690
4	0.053	0.880	0.775
5	0.041	0.921	0.846

7.2.1.1.e2 seabream provenance

In table 17 the fit parameters of the PCA performed on the dataset comprising the spectra of all the samples data based on the provenance of Orata are collected (364 variables for 64 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 17: Fit parameters of all the data about provenance of Orata in the 650-1000 cm^{-1} spectral range

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.431	0.431	0.003
2	0.236	0.667	0.393
3	0.173	0.841	0.701
4	0.058	0.899	0.806
5	0.040	0.939	0.878

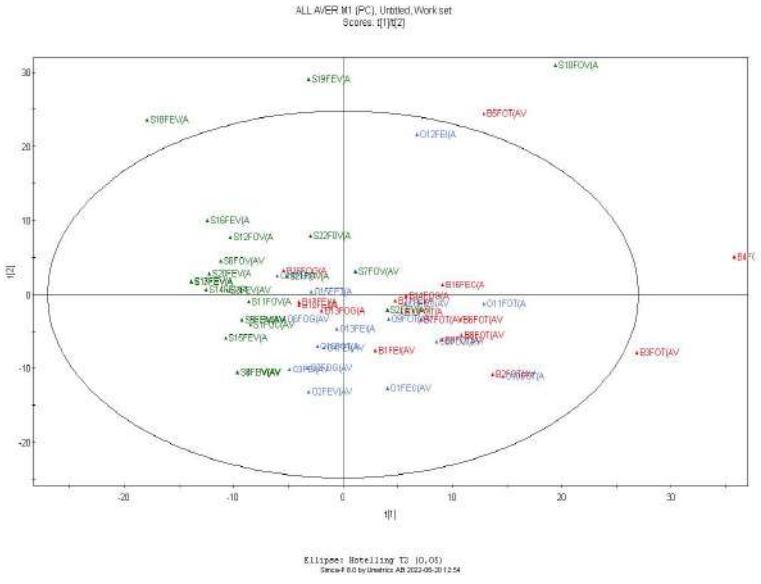
7.2.1.2 Analysis on the AVERAGED spectrum
7.2.1.2.a All species

In table 18 the fit parameters of the PCA performed on the dataset comprising the spectra of all the samples data collected (364 variables for 55 observations). The first five PCs account for 93.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. The components analysis for PC2 vs PC1 shows low level of omega3 range trent in the opposite right corner.

Table 18: Fit parameters of all the data in the 650-1000 cm^{-1} spectral range

Principal Component	explained variance	cumulative R^2	cumulative Q^2
1	0.316	0.316	0.285
2	0.267	0.583	0.536
3	0.148	0.730	0.675
4	0.094	0.824	0.777
5	0.056	0.880	0.842

Figure 25: PC2 vs PC1 scores plots of all the average samples data all three specimen in the 650-1000 cm^{-1} spectral range (seabass = blue, seabream = red, sardines = Green)



7.2.1.2.B1 Fresh

In table 19 the fit parameters of the PCA performed on the dataset comprising the spectra of all the fresh samples are collected (364 variables for 26 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, that are always close to the R^2 ones.

Table 19: Fit parameters of all the average data of Fresh Samples in the 650-1000 cm^{-1} spectral range.

Principal Component	explained variance	cumulative R^2	cumulative Q^2
1	0.324	0.324	0.197
2	0.275	0.599	0.479
3	0.178	0.776	0.685
4	0.076	0.852	0.776
5	0.045	0.897	0.824

Figure 26: PC2 vs PC(Right)1 and PC2 vs PC3(Left) scores plots of all the average Fresh samples data all three speciman in the 650-1000 cm^{-1} spectral range (seabass = blue, seabream = red, sardines = Green)

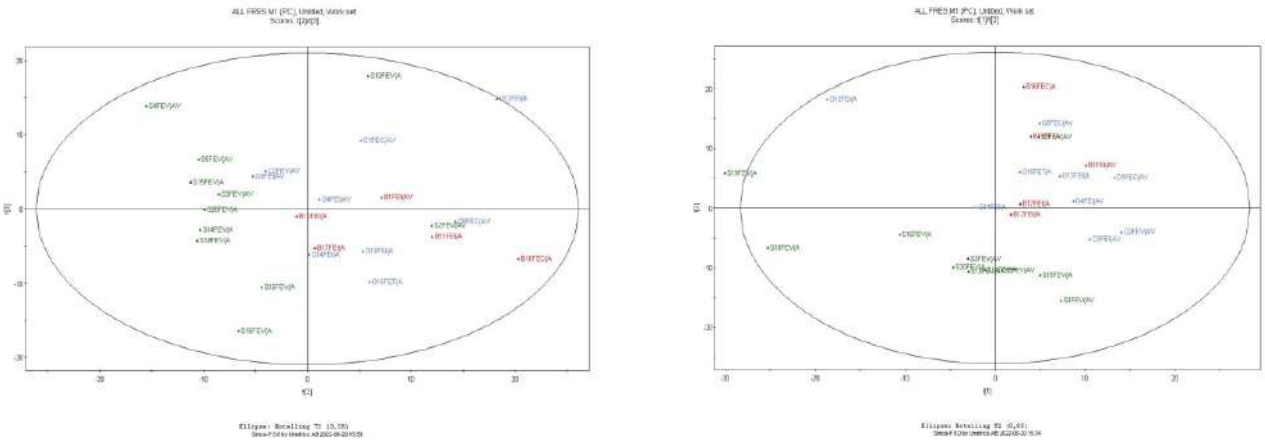
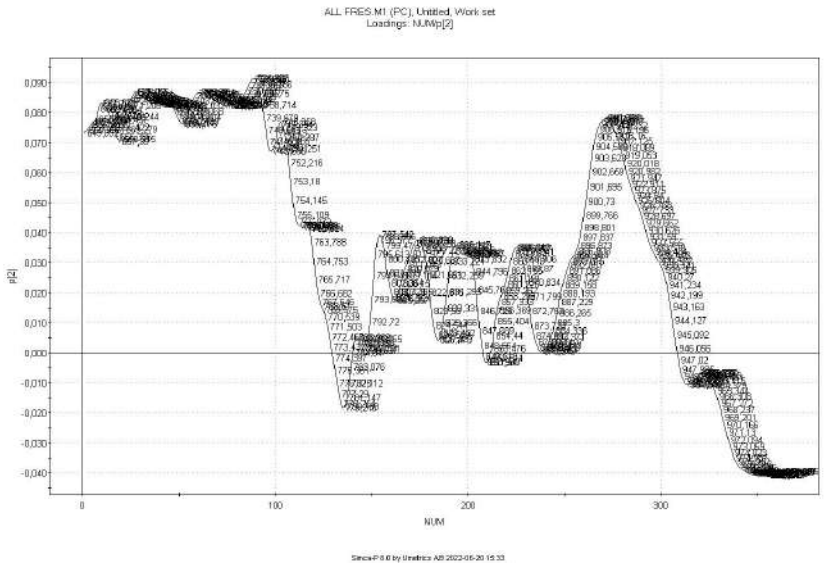


Figure 27: PC2 loading plots for an average Fresh of all data sample data in the 650-1000 cm^{-1} spectral range.



The loading plot of PC2, shows the omega 3 rich samples in the right upper corner. as shown in Figure 27, indicates that the absorptions in the range of approximately 650-1000 cm^{-1} can be correlated with positive values. Therefore, the samples are considered to be representative of omega-3 fatty acids.

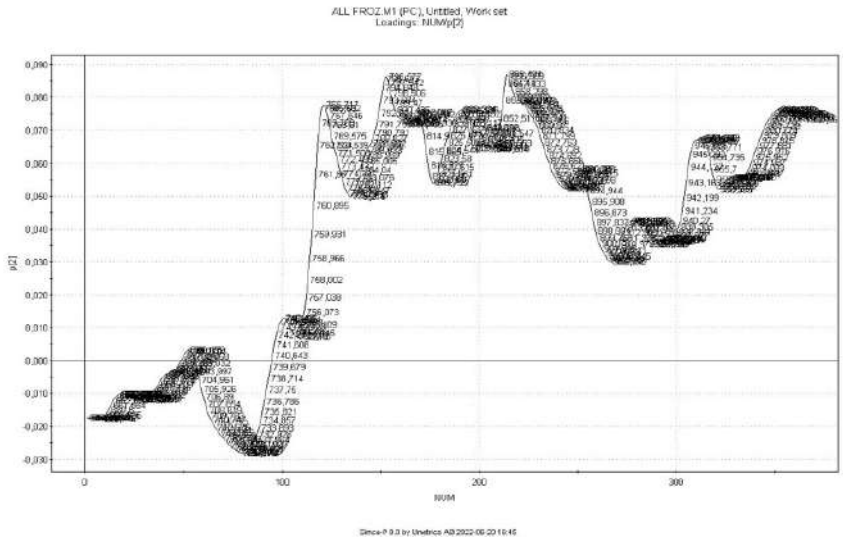
7.2.1.2.B2 Frozen

In table 20 the fit parameters of the PCA performed on the dataset comprising the spectra of all the frozen samples are collected (364 variables for 29 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, that are always close to the R^2 ones.

Table 20: Fit parameters of all the data of average Frozen samples in the 650-1000 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.323	0.323	0.256
2	0.262	0.585	0.491
3	0.187	0.772	0.692
4	0.096	0.868	0.802
5	0.040	0.908	0.851

Figure 28: PC2 loading plots for an average Frozen of all data sample data in the 650-1000 cm^{-1} spectral range.



The loading plot of PC2, shows the Omega3-rich samples are present in right upper corner of thr plane as shown in Figure 28, indicating that the absorptions in the range of approximately 650-1000 cm^{-1} can be correlated with positive values. Therefore, the samples are considered to be representative of omega-3 fatty acids.

7.2.1.2 B3 Seabass Vs Seabream

In table 21 the fit parameters of the PCA performed on the dataset comprising the spectra of all the Orata vs Branzino samples are collected (364 variables for 33 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, that are always close to the R^2 ones. These components do not show any particular Trent depending on Conservation.

Table 21: Fit parameters of all the data collection of Orata and Branzino in the 650-1000 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.353	0.353	0.285
2	0.270	0.5622	0.544
3	0.139	0.762	0.676
4	0.086	0.848	0.768
5	0.064	0.911	0.854

7.2.1.2.D1) Seabass Conservation

In Table These components do not show any particular Trent depending on Conservation.
22 the fit parameters of the PCA performed on the dataset comprising the spectra of all the conservation of Branzino samples are collected (364 variables for 17 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components do not show any particular Trent depending on Conservation.

Table 22: Fit parameters of all the branzino conservation data in the 650-1000 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.396	0.396	0.305
2	0.293	0.689	0.576
3	0.126	0.815	0.702
4	0.056	0.871	0.719
5	0.044	0.916	0.782

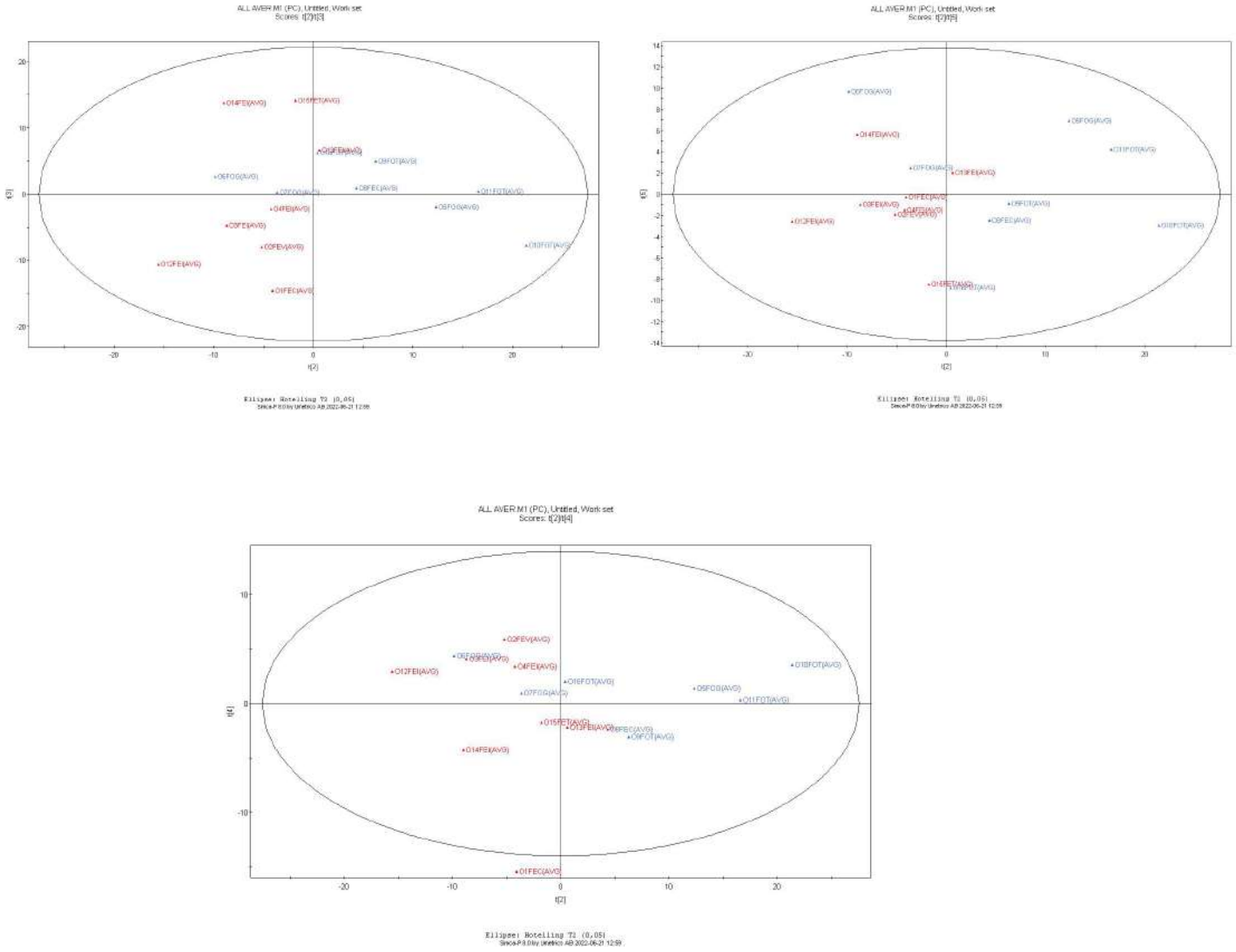
7.2.1.2.D2) Seabream Conservation

In table 23 the fit parameters of the PCA performed on the dataset comprising the spectra of all the conservation of orata samples are collected (364 variables for 16 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. Frozen orata (figure 29) seems to support this prediction: PC3 vs PC2, PC4 vs PC2, and PC5 vs PC2 scores plots should show omega3-rich samples in the right half of any plane with PC2 as the x-axis.

Table 23: Fit parameters of all the data of conservation of orata in the 650-1000 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.352	0.352	0.000
2	0.278	0.629	0.339
3	0.180	0.809	0.578
4	0.071	0.881	0.614
5	0.070	0.950	0.805

Figure 29: PC2 vs PC3(Left), PC2 vs PC5(Right) and PC2 vs PC4(Down) scores plots of all the average samples data for conservations of orata in the 650-1000 cm^{-1} spectral range (Fresh seabream = red, Frozen seabream=blue).



7.2.1.2.D3) Sardines Conservation

In table 24 the fit parameters of the PCA performed on the dataset comprising the spectra of all the conservation of sardine samples are collected (364 variables for 22 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components do not show any particular Trent depending on Conservation.

Table 24: Fit parameters of all the data for the conservation of sardine in the 650-1000 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.382	0.382	0.177
2	0.221	0.603	0.370
3	0.160	0.763	0.570
4	0.113	0.876	0.733
5	0.054	0.930	0.833

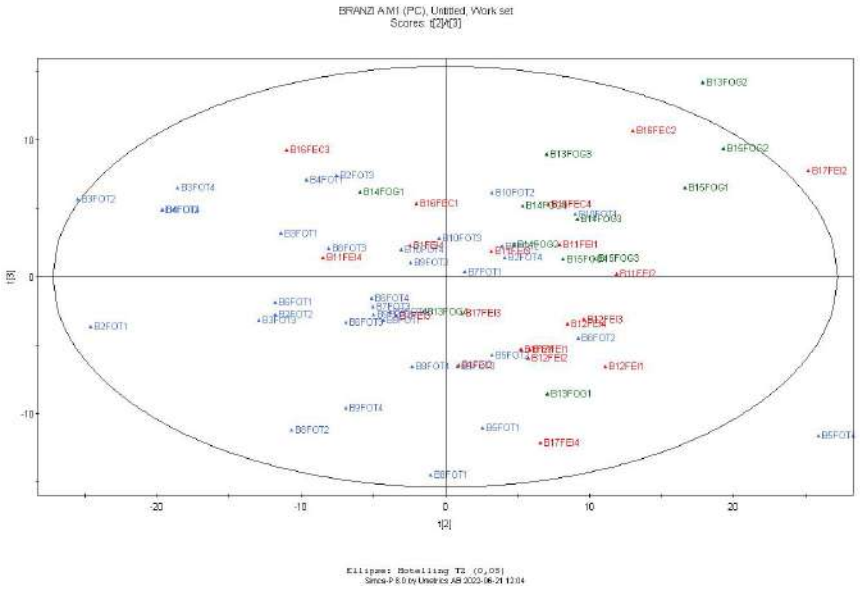
7.2.1.2.E1) Seabass Provenance

In table 25 the fit parameters of the PCA performed on the dataset comprising the spectra of all the Provenance of Branzino samples are collected (364 variables for 68 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. The comparison between PC3 vs PC2 demonstrates. The samples from Turkey exhibit a greater degree of richness in comparison to those from other regions.

Table 25: Fit parameters of all the data for the provenance of Branzino in the 650-1000 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.398	0.398	0.000
2	0.325	0.723	0.520
3	0.104	0.827	0.690
4	0.053	0.880	0.775
5	0.041	0.921	0.846

Figure 30: PC2 vs PC3 scores plots of all the average samples data for the provenance of Branzino in the 650-1000 cm^{-1} spectral range (Fresh seabass = red, Frozen seabass=blue,Grean).



7.2.1.2.E2) Seabream Provenance

In table 26 the fit parameters of the PCA performed on the dataset comprising the spectra of all the Provenance of Orata samples are collected (364 variables for 64 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components do not show any particular Trent depending on Conservation.

Table 26: Fit parameters of all the data for the provenance of Orata in the 650-1000 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.431	0.431	0.003
2	0.236	0.667	0.393
3	0.173	0.841	0.701
4	0.058	0.899	0.806
5	0.040	0.939	0.878

7.3.1 Spectral Range Of 1425-1770 Cm^{-1}

As highlighted in Section 6, this particular spectral range encompasses two highly concentrated bands that correspond to amide I and II, along with two absorptions that are relatively less intense at 1743 and 1458 cm-1, which are associated with fats and Esther triglycerides, respectively. The principal component analysis (PCA) demonstrated that:

7.3.1.1 PCA On ALL The Spectra

7.3.1.1.a All Species

In Table 27 the fit parameters of the PCA performed on the dataset comprising all the spectra are collected (359 variables for 220 observations). The first five PCs account for 99.1% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. No significant grouping occurred in the corresponding scores plot regarding the fish species, the conservation treatment and the provenance.

Table 27: Fit parameters of all the data in the 1425-1770 cm^{-1} spectral range

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.812	0.812	0.810
2	0.138	0.950	0.949
3	0.026	0.976	0.974
4	0.008	0.984	0.990
5	0.007	0.991	0.993

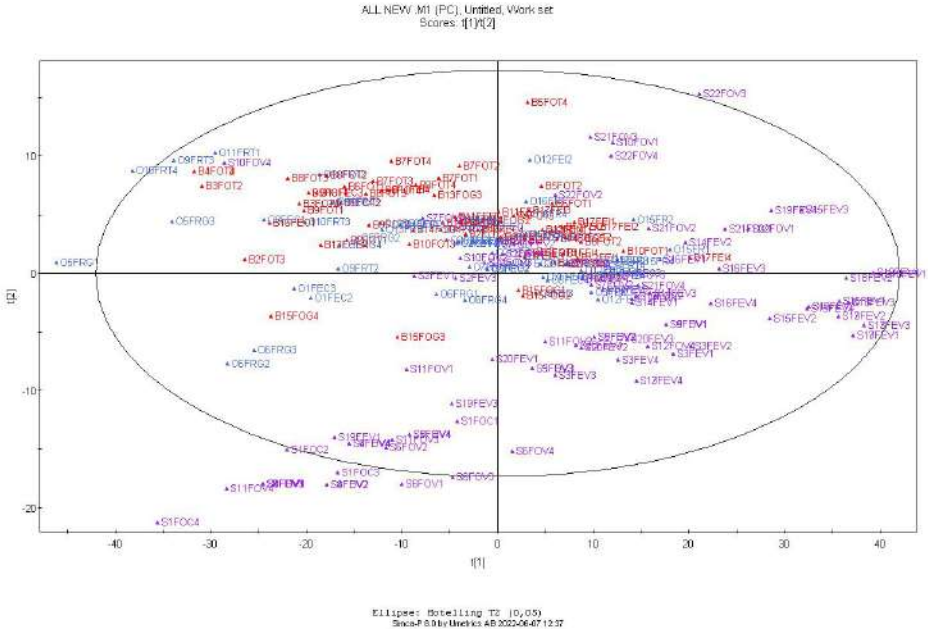
7.3.1.1.B1 Fresh

In table 28 the fit parameters of the PCA performed on the dataset comprising the spectra of all the fresh samples are collected (359 variables for 96 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. All the scores plots taken into account, the PC2 vs PC1 one shows a grouping of the representative points of the sardines' spectra in the lower right corner (see Figure 29).

Table 28: Fit parameters of the fresh samples data in the 1425-1770 cm^{-1} spectral range

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.818	0.818	0.812
2	0.122	0.940	0.937
3	0.029	0.970	0.959
4	0.012	0.982	0.974
5	0.008	0.990	0.985

Figure 31: PC2 vs PC1 scores plots of all the fresh samples data in the 1425-1770 cm^{-1} spectral range (seabass = Blue, seabream = Red, sardines = Purple).



The PC1 loading plot (Figure 32 left) shows that negative values can be associated with the absorptions at about 1458 and 1743 cm^{-1} while the PC2 loading plot (figure 32 right) shows that the same absorptions contribute to positive values; thus samples representative "fat" samples should fall in the left upper corner of the (PC1, PC2) plane, leaving the leaner ones (that is sardines) in the opposite position, in acceptable agreement with the results shown in figure 49.

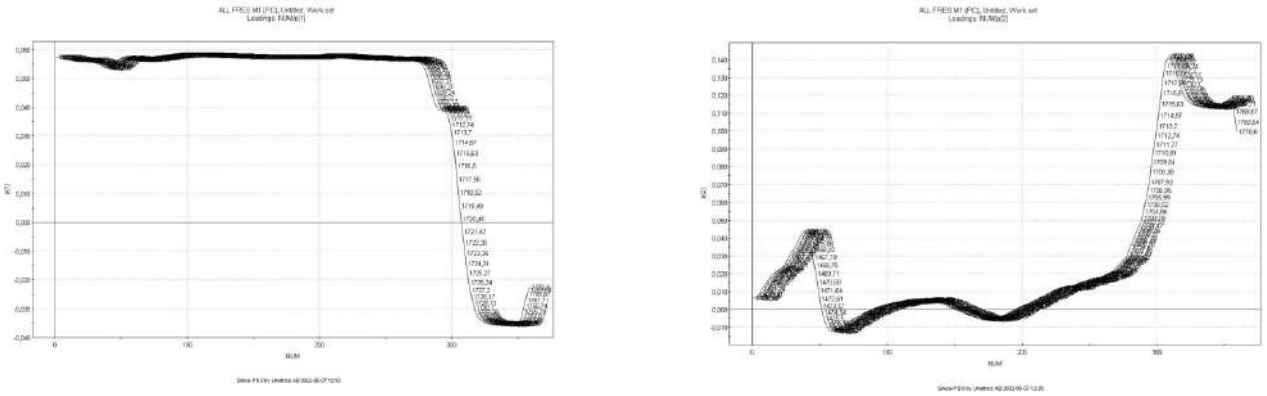


Figure 32: PC1 (left) and PC2 (right) loading plots of all the fresh samples data in the 1425-1770 cm^{-1} spectral range

7.3.1.1.b2 Frozen

In Table 29 the fit parameters of the PCA performed on the dataset comprising the spectra of all the frozen samples are collected (359 variables for 120 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 29: Fit parameters of the frozen samples data in the 1425-1770 cm^{-1} spectral range

Principal .Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.804	0.804	0.801
2	0.156	0.960	0.959
3	0.023	0.983	0.982
4	0.006	0.989	0.988
5	0.003	0.992	0.991

All the scores plots taken into account, two show a grouping of the representative points of the sardines' spectra: PC2 vs PC1 in the lower right corner (see figure 33 left) and PC3 vs PC2 in the upper left one (see figure 33 right).

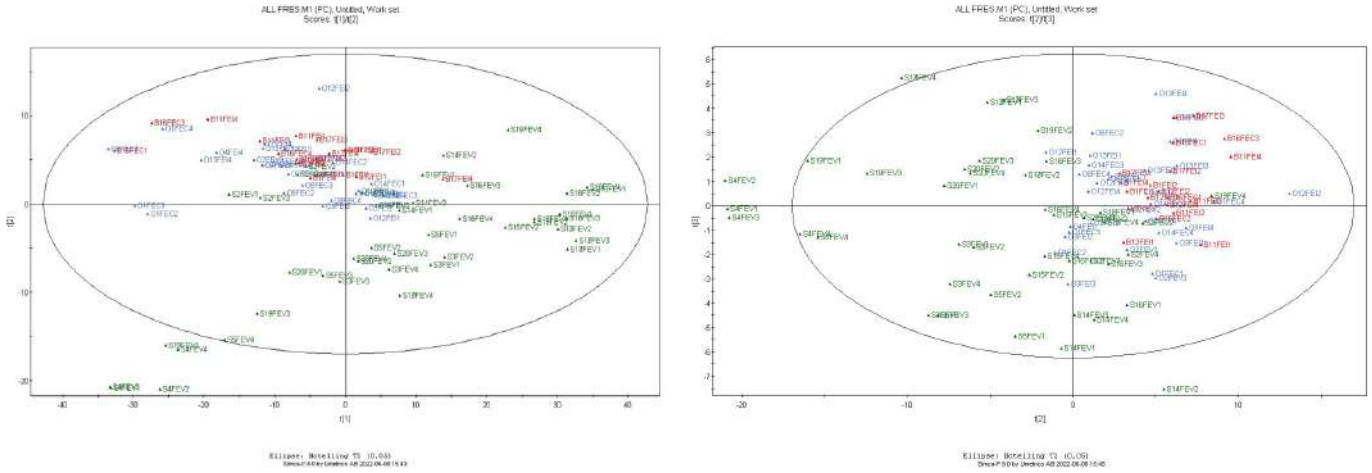


Figure 33: PC2 vs PC1 (left) and PC3 vs PC2 (right) scores plots of all the frozen samples data in the 1425-1770 cm^{-1} spectral range (seabass = blue, seabream = red, sardines = green)

The PC1 loading plot (figure 34 left) shows that negative values can be associated to the absorptions at about 1458 and 1743 cm^{-1} while the PC2 loading plot (figure 52 right) shows that the same absorptions contribute to positive values; thus, samples representative "fat" samples should fall in the left upper corner of the (PC1, PC2) plane, leaving the leaner ones (that is sardines) in the opposite position.

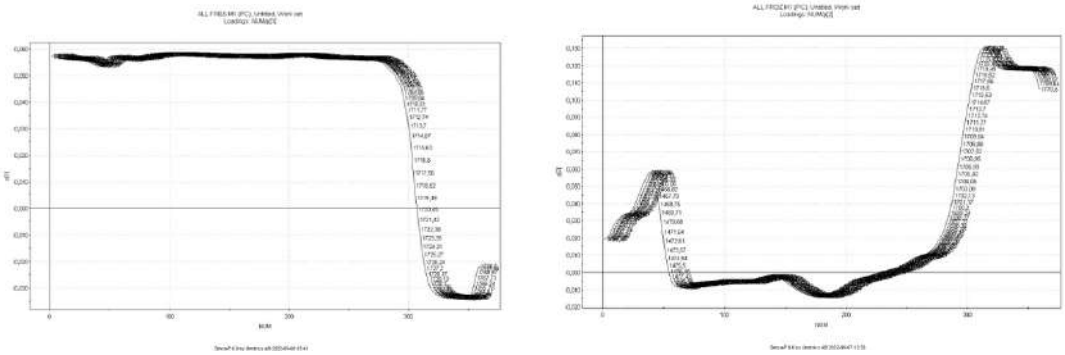
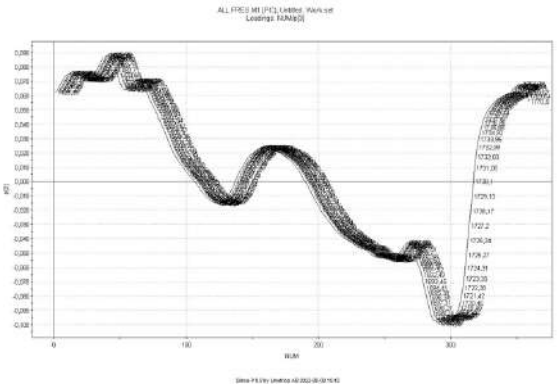


Figure 34: PC1 (left) and PC2 (right) loading plots of all the frozen samples data in the 1425-1770 cm^{-1} spectral range

On the other hand, considering that the PC3 loading plot (figure 33) shows that the absorption located at about 1743 cm^{-1} contribute to negative values, it is possible to resume that samples representative "fat" samples should fall in the lower right corner of the (PC2, PC3) plane, leaving the leaner ones (that is sardines) in the opposite position.

Figure 35: PC3 loading plot of all the frozen samples data in the 1425-1770 cm^{-1} spectral range.



7.3.1.1.C Seabass Vs Seabream

In Table 30 the fit parameters of the PCA performed on the dataset comprising the spectra of all the Orata and Branzino samples are collected (359 variables for 132 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components do not exhibit any visible trend with respect to conservation.

Table 30: Fit parameters of Orata and Branzino samples data in the 1425-1770 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.873	0.873	0.871
2	0.079	0.951	0.949
3	0.025	0.976	0.975
4	0.010	0.986	0.985
5	0.005	0.991	0.991

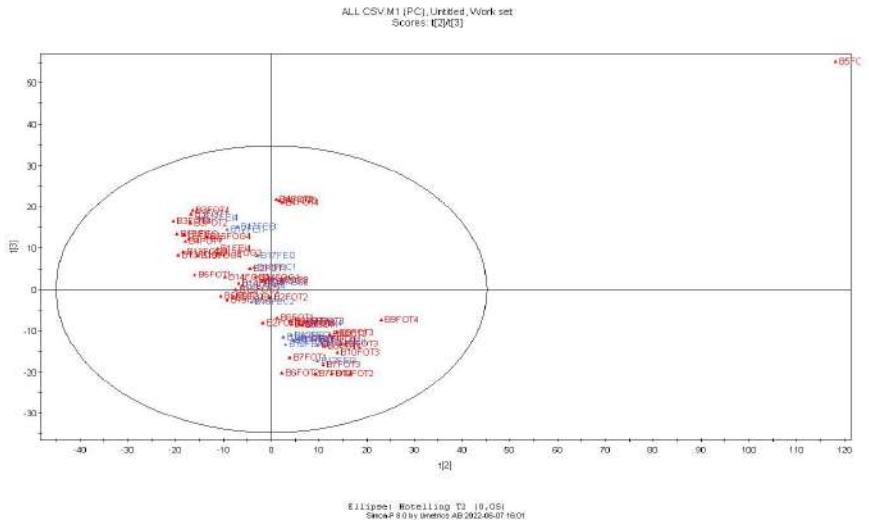
7.3.1.1.D1 Seabass Conservation

In Table 31 the fit parameters of the PCA performed on the dataset comprising the spectra of all the conservation of seabream samples are collected (266 variables for 68 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2ones. PC2 vs PC3 scores plots exabit the fish from Greece occupied the lower corner in the plane.

Table 31: Fit parameters of the frozen samples data in the 1425-1770 cm^{-1} spectral range

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.640	0.640	0.629
2	0.122	0.761	0.629
3	0.072	0.833	0.735
4	0.056	0.889	0.817
5	0.028	0.917	0.858

Figure 36: PC2 vs PC3 scores plots of all the samples data of Conservation of seabass in the 1425-1770 cm^{-1} spectral range (seabass Fresh= blue, seabass Frozen = red).



7.3.1.1.d2 seabream conservation

In Table 32 the fit parameters of the PCA performed on the dataset comprising the spectra of all the conservation of seabream samples are collected (359 variables for 64 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components do not exhibit any visible trend with respect to conservation.

Table 32: Fit parameters of the samples data of conservation of branzino in the 1425-1770 cm^{-1} spectral range

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.882	0.882	0.874
2	0.078	0.960	0.956
3	0.021	0.981	0.973
4	0.009	0.990	0.988
5	0.004	0.994	0.992

7.3.1.1.d3 sardines conservation

In Table 33 the fit parameters of the PCA performed on the dataset comprising the spectra of all the conservation of sardine samples are collected (359 variables for 64 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components do not exhibit any visible trend with respect to conservation.

Table 33: Fit parameters of the samples data of conservation of Sardine in the 1425-1770 cm^{-1} spectral range.

Principal Component	explained variance	cumulative R^2	cumulative Q^2
1	0.791	0.791	0.787
2	0.157	0.949	0.946
3	0.024	0.972	0.968
4	0.012	0.984	0.979
5	0.006	0.990	0.987

7.3.1.1.e1) seabass provenance

In Table 34 the fit parameters of the PCA performed on the dataset comprising the spectra of all the conservation of sardine samples are collected (359 variables for 55 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components do not exhibit any visible trend with respect to conservation.

Table 34: Fit parameters of the branzino provenance samples data in the 1425-1770 cm^{-1} spectral range

Principal Component	explained variance	cumulative R^2	cumulative Q^2
1	0.882	0.882	0.879
2	0.072	0.954	0.949
3	0.022	0.976	0.973
4	0.008	0.984	0.982
5	0.006	0.991	0.989

7.3.1.1.e2) seabream provenance

In Table 35 the fit parameters of the PCA performed on the dataset comprising the spectra of all the provenance of Orata samples are collected (359 variables for 65observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components do not exhibit any visible trend with respect to conservation.

Table 35: Fit parameters of the provenance of Orata samples sample data in the 1425-1770 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.883	0.882	0.874
2	0.078	0.960	0.956
3	0.021	0.981	0.978
4	0.009	0.990	0.988
5	0.004	0.994	0.992

7.3.1.2 Analysis on The AVERAGED Spectra

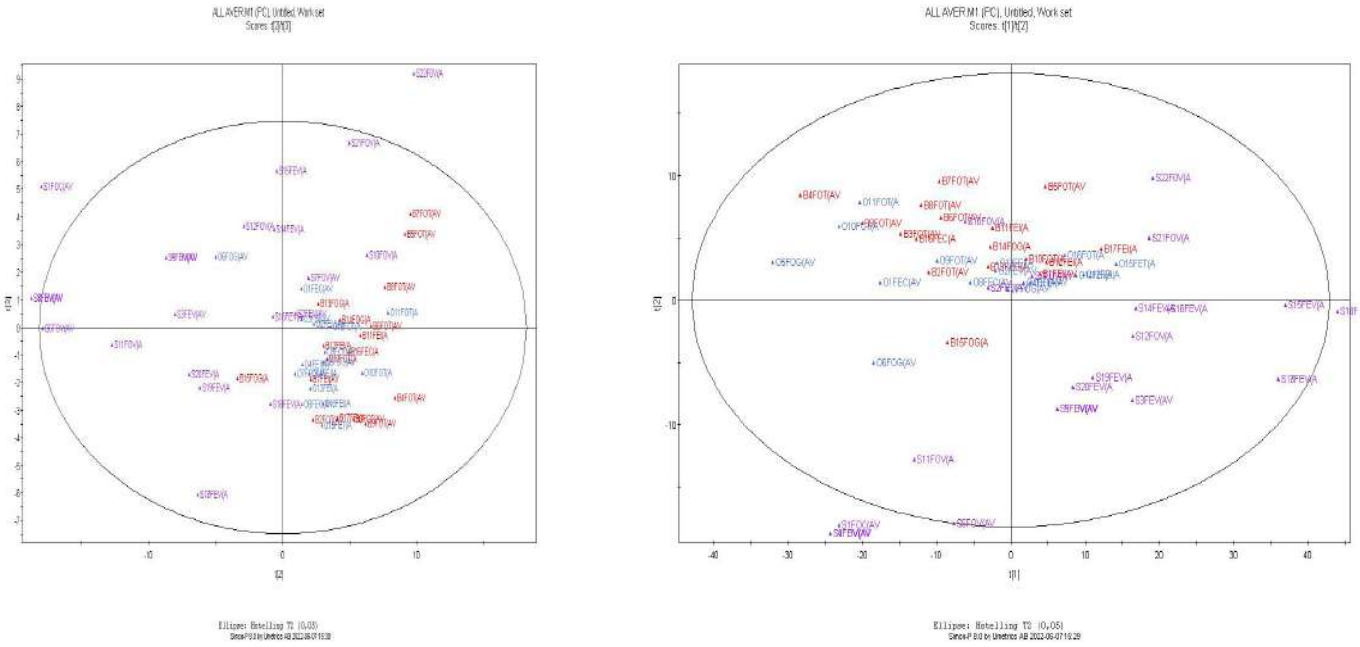
7.3.1.2.A Species

In Table 36 the fit parameters of the PCA performed on the dataset comprising the spectra of all the samples are collected (359 variables for 55observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. PC2 vs PC1 same as before shows Lean Sardines in the lower right corner.PC3 vs PC2 same as before shows Lean Sardines in the upper left corner.

Table 36: Fit parameters of the average samples data in the 1425-1770 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.810	0.810	0.793
2	0.145	0.955	0.950
3	0.025	0.980	0.976
4	0.008	0.987	0.984
5	0.005	0.993	0.994

Figure 37: PC2 vs PC3 and PC2 vs PC1 scores plots of all the samples data of the 1425-1770 cm^{-1} spectral range (seabass = blue, seabream Frozen = red sardine=Green).



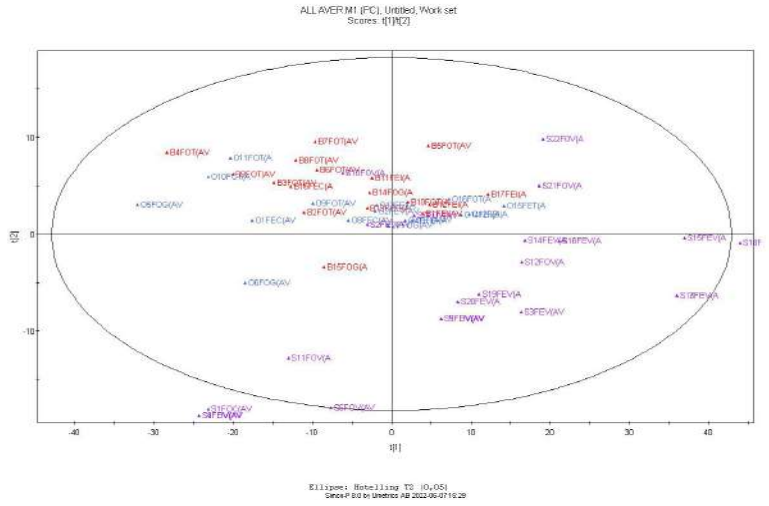
7.3.1.2.B1 Fresh

In Table 38 the fit parameters of the PCA performed on the dataset comprising the spectra of all the samples are collected (359 variables for 55observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. PC2 vs PC1 same as before shows Lean Sardines in the lower right corner.

Table 38: Fit parameters of the fresh samples data in the 1425-1770 cm^{-1} spectral rang

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.826	0.826	0.780
2	0.123	0.949	0.933
3	0.026	0.975	0.962
4	0.013	0.988	0.979
5	0.005	0.993	0.987

Figure 38: PC2 vs PC1 scores plots of all the samples data of the 1425-1770 cm^{-1} spectral range (seabass = blue, seabream Frozen = red sardine=Green).



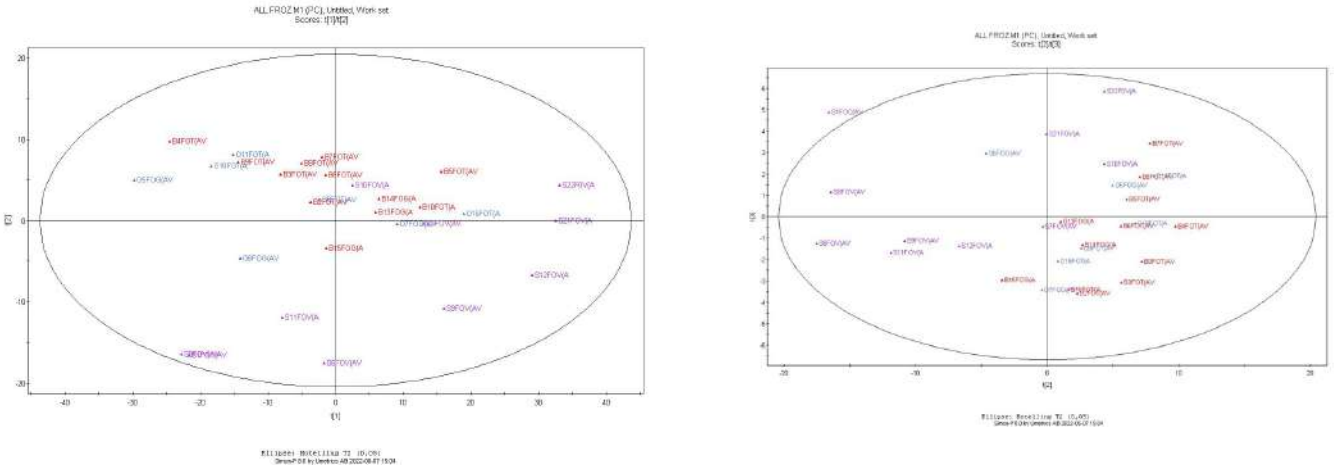
7.3.1.2.B2 Frozen

In Table 39 the fit parameters of the PCA performed on the dataset comprising the spectra of all the Frozen samples are collected (359 variables for 55observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. PC2 vs PC1 same as before shows Lean Sardines in the lower right corner, PC3 vs PC2 same as before shows Lean Sardines in the upper left corner.

Table 39: Fit parameters of the frozen samples data in the 1425-1770 cm^{-1} spectral range

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.794	0.794	0.777
2	0.173	0.967	0.962
3	0.019	0.986	0.982
4	0.005	0.990	0.987
5	0.004	0.994	0.992

Figure 39: PC2 vs PC1 and PC2 vs PC3 scores plots of all the frozen samples data of the 1425-1770 cm^{-1} spectral range (seabass = blue, seabream Frozen = red sardine=Green).



7.3.1.2.c seabass vs seabream

In Table 40 the fit parameters of the PCA performed on the dataset comprising the spectra of all comparison study for orate and branzino samples are collected (359 variables for 55observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components do not exhibit any visible trend with respect to conservation.

Table 40: Fit parameters for the comparison study for orate and branzino samples data in the 1425-1770 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.850	0.850	0.843
2	0.090	0.940	0.927
3	0.033	0.973	0.966
4	0.011	0.984	0.977
5	0.004	0.990	0.985

7.3.1.2.D1 Seabass Conservation

In table 41 the fit parameters of the PCA performed on the dataset comprising the spectra of all the Frozen samples are collected (359 variables for 17observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components do not exhibit any visible trend with respect to conservation.

Table 41: Fit parameters of the branzino conservation of samples data in the 1425-1770 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.856	0.856	0.842
2	0.088	0.944	0.920
3	0.030	0.974	0.957
4	0.010	0.984	0.962
5	0.007	0.991	0.975

7.3.1.2.D2 Seabream Conservation

In Table 42 the fit parameters of the PCA performed on the dataset comprising the spectra of all the samples based on conservation of orata are collected (356 variables for 16 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components do not exhibit any visible trend with respect to conservation.

Table 42: Fit parameters of conservation of orata samples data in the 1425-1770 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.875	0.875	0.853
2	0.077	0.953	0.927
3	0.028	0.981	0.968
4	0.008	0.989	0.979
5	0.004	0.994	0.982

7.3.1.2.D3 Sardines Conservation

In Table 43 the fit parameters of the PCA performed on the dataset comprising the spectra of all the samples based on conservation of sardin are collected (359 variables for 22 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.
Table 43: Fit parameters of the conservation of sardin samples data in the 1425-1770 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.801	0.801	0.776
2	0.160	0.961	0.951
3	0.020	0.981	0.970
4	0.009	0.990	0.983
5	0.004	0.994	0.987

7.3.1.2.E1 Seabass Provenance

In Table 44 the fit parameters of the PCA performed on the dataset comprising the spectra of all the samples based on Provenance of Branzino are collected (359 variables for 68 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components do not exhibit any visible trend with respect to conservation.

Table 44: Fit parameters of Provenance of Branzino samples data in the 1425-1770 cm^{-1} spectral range

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.882	0.882	0.879
2	0.072	0.954	0.949
3	0.022	0.976	0.973
4	0.006	0.984	0.982
5	0.004	0.991	0.989

7.3.1.2.E2 Seabream Provenance

In Table 45 the fit parameters of the PCA performed on the dataset comprising the spectra of all the samples based on Provenance of Orata are collected (359 variables for 64 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 45: Fit parameters of theorata samples data in the 1425-1770 cm^{-1} spectral range

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.882	0.882	0.874
2	0.078	0.960	0.956
3	0.021	0.981	0.978
4	0.009	0.990	0.988
5	0.004	0.994	0.992

7.4.1 2700-3100 Cm^{-1} Spectral Range

7.4.1.1 Analysis on All the Spectra

7.4.1.1.A Species

In Table 46 the fit parameters of the PCA performed on the dataset comprising the spectra of all the samples are collected (416 variables for 220 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. These components do not exhibit any visible trend with respect to conservation.

Table 46: Fit parameters of the all-samples data in the 2700-3100 cm^{-1} spectral range

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.835	0.835	0.835
2	0.144	0.979	0.979
3	0.016	0.996	0.995
4	0.003	0.998	0.998
5	0.001	0.999	0.999

7.4.1.1.B1 Fresh

In Table 47 the fit parameters of the PCA performed on the dataset comprising the spectra of all the Fresh samples are collected (416 variables for 220 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. PC2 vs PC1 shows SARDINES in the lower right corner, PC3 vs PC2 shows Sardines in the lower right corner.

Table 47: Fit parameters of the fresh samples data in the 2700-3100 cm^{-1} spectral range

Principal Component	explained variance	cumulative R^2	cumulative Q^2
1	0.846	0.846	0.844
2	0.129	0.975	0.974
3	0.022	0.997	0.997
4	0.002	0.999	0.999
5	0.000	0.999	0.999

Figure 40: PC2 vs PC1(Right) and PC2 vs PC3(Left) scores plots of all the Fresh samples data of the 2700-3100 cm^{-1} spectral range (seabass = blue, seabream, Frozen = red, sardine=Green).

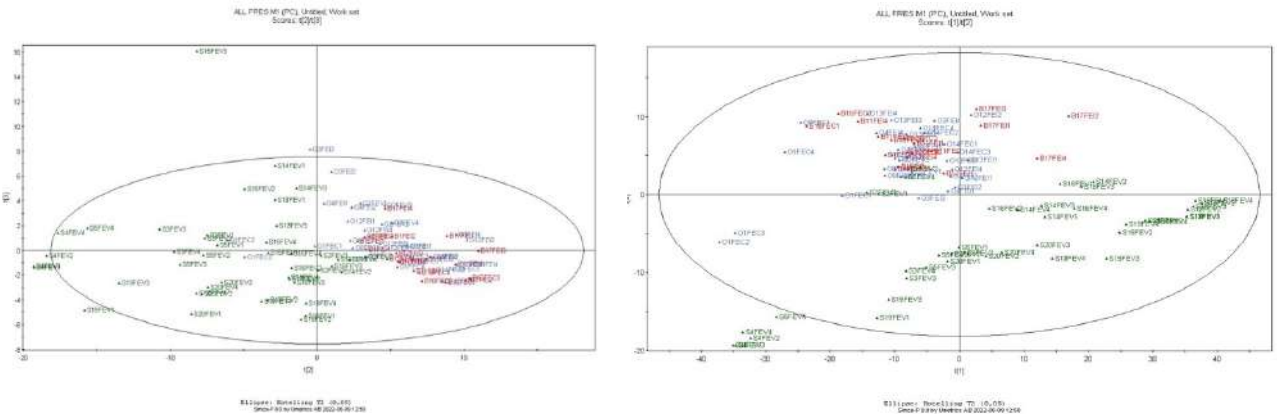
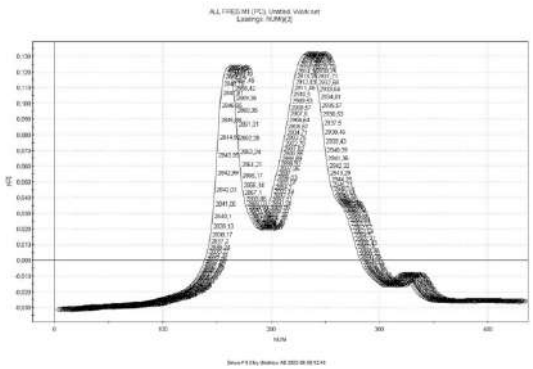


Figure 41: PC2 loading plots of all the fresh samples data in the 2700-3100 cm^{-1} spectral range



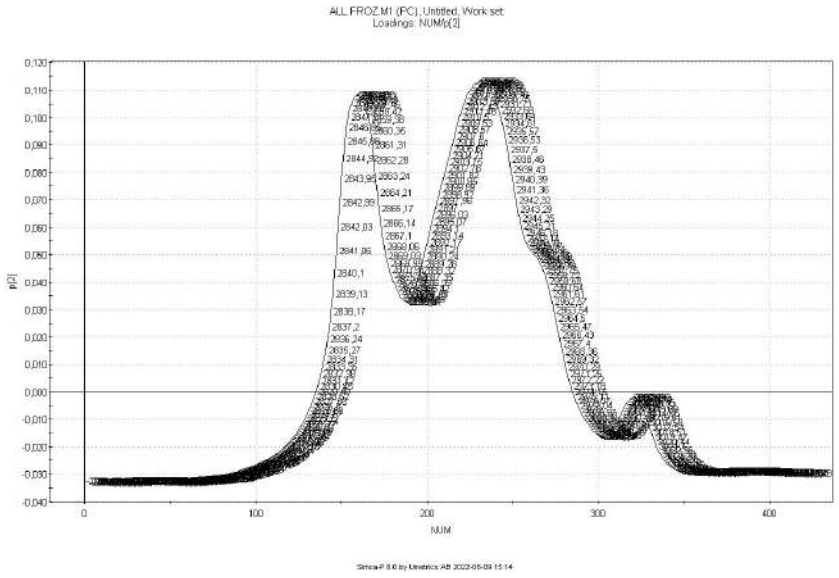
7.4.1.1.B2 Frozen

In Table 48 the fit parameters of the PCA performed on the dataset comprising the spectra of all the Frozen samples are collected (416 variables for 120 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 48: Fit parameters of the frozen samples data in the 2700-3100 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.813	0.813	0.811
2	0.167	0.979	0.979
3	0.014	0.994	0.993
4	0.004	0.998	0.998
5	0.001	0.999	0.999

Figure 42: PC2 vs PC5 scores plots of all the frozen samples data of the 2700-3100 cm^{-1} spectral range (seabass = blue, seabream ,Frozen = red ,sardine=Green).



7.4.1.1.C Seabass Vs Seabream

In Table 49 the fit parameters of the PCA performed on the dataset comprising the spectra of all the differences between Orata and Branzino the are collected (416 variables for 132 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 49: Fit parameters of the difference between Orata and Branzino samples data in the 2700-3100 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.844	0.844	0.841
2	0.125	0.969	0.968
3	0.020	0.989	0.988
4	0.007	0.996	0.996
5	0.002	0.998	0.998

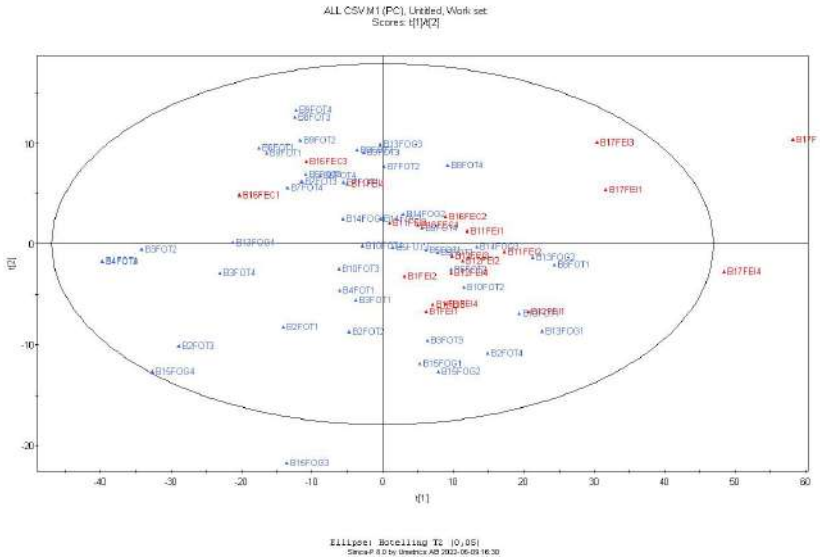
7.4.1.1.D1 Seabass Conservation

In Table 48 the fit parameters of the PCA performed on the dataset comprising the spectra date based on conservation on Branzino of all the are collected (416 variables for 68 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 48: Fit parameters of the Branzino conservation samples data in the 2700-3100 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.845	0.845	0.839
2	0.123	0.968	0.964
3	0.021	0.989	0.986
4	0.006	0.995	0.994
5	0.003	0.998	0.997

Figure 43: PC1 vs PC2 scores plots of all the conservation of Branzino in a samples data of the 2700-3100 cm^{-1} spectral range (seabass = blue, seabream = red, sardine=Green).



7.4.1.1.d2 seabream conservation

In Table 49 the fit parameters of the PCA performed on the dataset comprising the spectra date based on conservation on orata of all the are collected (416 variables for 64 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 49: Fit parameters of the conservation data of Orata in the 2700-3100 cm^{-1} spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.853	0.854	0.842
2	0.120	0.975	0.972
3	0.017	0.992	0.991
4	0.006	0.998	0.997
5	0.001	0.999	0.998

7.4.1.1.D3) Sardines Conservation

In Table 50 the fit parameters of the PCA performed on the dataset comprising the spectra date based on conservation on Sardine of all the are collected (416 variables for 87 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 50: Fit parameters of the conservation on Sardine samples data in the 2700-3100 cm^{-1} spectral range.

Principal Component	explained variance	cumulative R^2	cumulative Q^2
1	0.935	0.935	0.934
2	0.044	0.979	0.978
3	0.020	0.998	0.998
4	0.001	1.000	1.000
5	0.000	1.000	1.000

7.4.1.1.E1) Seabass Provenance

In Table 51 the fit parameters of the PCA performed on the dataset comprising the spectra date based on the Provenance of Branzino are all the are collected (416 variables for 68 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 51: Fit parameters of the branzino Provenance base samples data in the 2700-3100 cm^{-1} spectral range.

Principal Component	explained variance	cumulative R^2	cumulative Q^2
1	0.845	0.845	0.839
2	0.123	0.968	0.964
3	0.021	0.989	0.986
4	0.006	0.995	0.994
5	0.003	0.998	0.997

7.4.1.1.E2) Seabream Provenance

In Table 52 the fit parameters of the PCA performed on the dataset comprising the spectra date based on the Provenance of orate are all the are collected (416 variables for 64 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 52: Fit parameters of the Orata Provenance samples data in the 2700-3100 cm^{-1} spectral range.

Principal Component	explained variance	cumulative R^2	cumulative Q^2
1	0.854	0.854	0.842
2	0.120	0.975	0.972
3	0.017	0.992	0.991
4	0.006	0.998	0.997
5	0.001	0.999	0.998

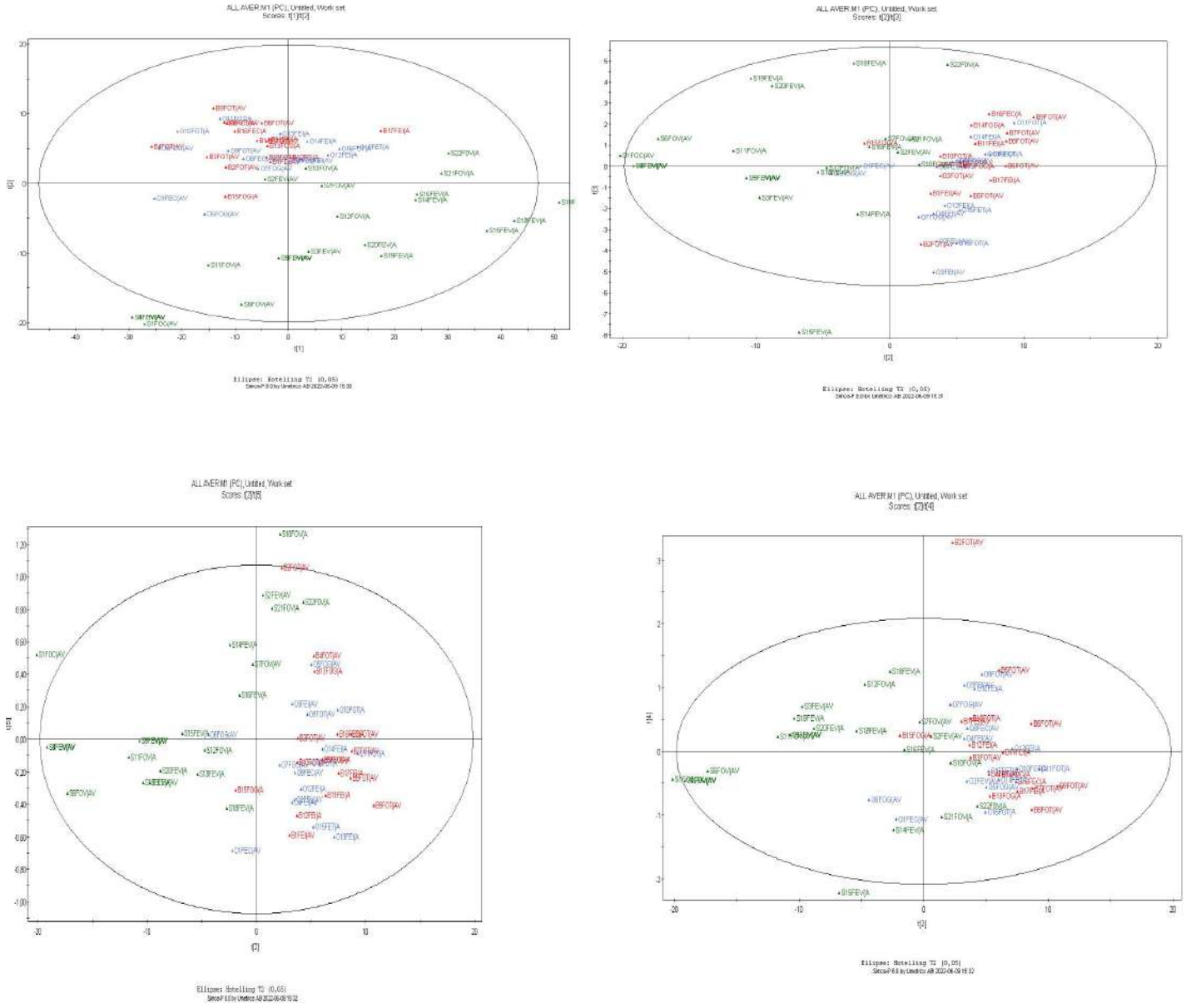
7.4.1.2) Analysis of The AVERAGED Spectra
7.4.1.2.A) Species

In Table 53 the fit parameters of the PCA performed on the dataset comprising the spectra date of all averages the are collected (416 variables for 55 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones. PC2 vs PC1 shows SARDINES in the lower right corner,PC3 vs PC2 shows SARDINES in the left halfplane, PC4 vs PC2 shows SARDINES in the left halfplane,PC5 vs PC2 shows SARDINES in the left halfplane

Table 53: Fit parameters of all the average samples data in the 2700-3100 cm^{-1}spectral range.

Principal Component	explained variance	cumulative R^2	cumulative Q^2
1	0.835	0.835	0.828
2	0.149	0.985	0.984
3	0.012	0.997	0.997
4	0.002	0.999	0.998
5	0.000	0.999	0.999

Figure 44: PC1 vs PC2(Left), PC2 vs PC3(Right), PC4 vs PC2 and PC5(Down right) vs PC2(Down left) scores plots for all the average samples data of the 2700-3100 cm^{-1} spectral range (seabass = blue, seabream = red, sardine=Green).



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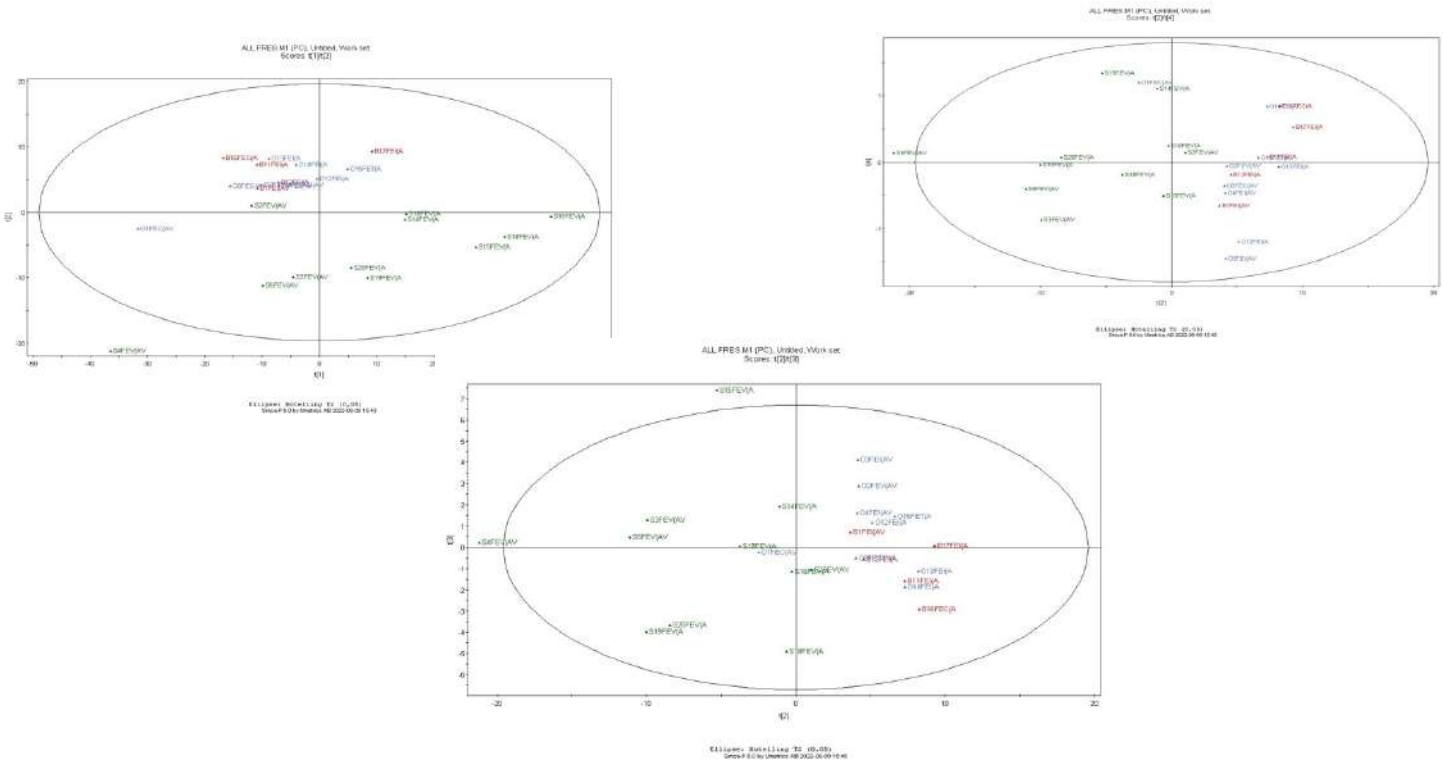
7.4.1.2.B1 Fresh

In Table 54 the fit parameters of the PCA performed on the dataset comprising the spectra date of Fresh averages samples are collected (416 variables for 26 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 54: Fit parameters of all the Fresh average samples data in the 2700-3100 cm^{-1}spectral range.

Principal Component	explained variance	cumulative R^2	cumulative Q^2
1	0.847	0.847	0.821
2	0.136	0.982	0.979
3	0.016	0.998	0.997
4	0.001	0.999	0.998
5	0.000	0.999	0.999

Figure 45: PC1 vs PC2(right) , PC3vs PC2(left) and PC5 vs PC2(down) scores plots for all the Fresh average samples data of the 2700-3100 cm^{-1} spectral range (seabass = blue, seabream = red, sardine=Green).



7.4.1.2.b2) frozen

In Table 55 the fit parameters of the PCA performed on the dataset comprising the spectra date of Frozen averages samples are collected (416 variables for 29 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 55: Fit parameters of all the Frozen average samples data in the 2700-3100 cm^{-1}spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.802	0.802	0.790
2	0.182	0.984	0.982
3	0.012	0.996	0.994
4	0.002	0.998	0.997
5	0.001	0.999	0.998

7.4.1.2.C) Seabass Vs Seabream

In Table 56 the fit parameters of the PCA performed on the dataset comprising the spectra date of comparison between Orata and branzino samples are collected (416 variables for 32 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 56: Fit parameters of the comparison between Orata and branzino average samples data in the 2700-3100 cm^{-1}spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.866	0.866	0.859
2	0.106	0.973	0.969
3	0.020	0.993	0.990
4	0.004	0.997	0.995
5	0.001	0.998	0.997

7.4.1.2.D1 Seabass Conservation

In Table 57 the fit parameters of the PCA performed on the dataset comprising the spectra date conservation of seabass samples are collected (416 variables for 32 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 57: Fit parameters of the conservation of seabass average samples data in the 2700-3100 cm^{-1}spectral range

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.853	0.853	0.843
2	0.116	0.969	0.950
3	0.024	0.993	0.987
4	0.003	0.996	0.992
5	0.002	0.998	0.995

7.4.1.2.D2) Seabream Conservation

In Table 58 the fit parameters of the PCA performed on the dataset comprising the spectra date conservation of sea bream samples are collected (416 variables for 16 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 58: Fit parameters of the conservation of seabass average samples data in the 2700-3100 cm^{-1}spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.891	0.891	0.868
2	0.089	0.980	0.972
3	0.015	0.995	0.991
4	0.003	0.998	0.996
5	0.001	0.999	0.997

7.4.1.2.D3 Sardines Conservation

In Table 59 the fit parameters of the PCA performed on the dataset comprising the spectra date conservation of sardine samples are collected (416 variables for 87 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 59: Fit parameters of the conservation of Sardine average samples data in the 2700-3100 cm^{-1}spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.935	0.935	0.934
2	0.044	0.979	0.978
3	0.020	0.998	0.998
4	0.001	1.000	1.000
5	0.000	1.000	1.000

7.4.1.2.E1) Seabass Provenance

In Table 60 the fit parameters of the PCA performed on the dataset comprising the spectra date Provenance of sea bass samples are collected (416 variables for 68 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 60: Fit parameters of the provenance of sea bass average samples data in the 2700-3100 cm^{-1}spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.845	0.845	0.839
2	0.123	0.968	0.964
3	0.021	0.989	0.986
4	0.006	0.995	0.994
5	0.003	0.998	0.997

7.4.1.2.E2 Seabream Provenance

In Table 61 the fit parameters of the PCA performed on the dataset comprising the spectra date Provenance of sea bass samples are collected (416 variables for 64 observations). The first five PCs account for 99.0% of the total variance of the data, and the good quality of the fit is reflected in the Q^2 values, which are always close to the R^2 ones.

Table 61: Fit parameters of the provenance of sea bass average samples data in the 2700-3100 cm^{-1}spectral range.

Principal Component	**explained variance**	**cumulative R^2**	**cumulative Q^2**
1	0.854	0.854	0.842
2	0.120	0.975	0.972
3	0.017	0.992	0.991
4	0.006	0.998	0.997
5	0.001	0.999	0.998

8. Conclusions

This study showcases the application of MIR spectroscopy in acquiring spectra for distinct behavioral patterns of fresh and frozen, as well as wild and farmed, fish. This information can be utilized to evaluate essential data and gain a comprehensive understanding of the sample's nature and chemical composition. According to the results of our spectral analysis, it was determined that the spectral differences observed among the orata, branzino, and sardine samples were not conducive to the study of these particular species. In order to comprehend the chemical composition of the sample being analyzed with the aid of chemometric techniques,

The variation between fresh and frozen fish was examined with respect to their protein and fat composition. Orata and branzino are classified as oily fish. Simultaneously, sardines are piscine species that possess a low-fat content. Both fresh and frozen fish are abundant in protein and fat, specifically omega-3 fatty acids, which are present in both Orata and branzino. The fresh fish have been marked spectra that are nearly identical to those of the frozen fish. The disparity between fresh and frozen sardines has been instrumental in clarifying the distinctions between fresh and frozen.

In conclusion, the examination of a significant number of samples can enable the enhancement of consumer protection against adulterations and fraudulent claims. In conclusion, the utilization of IR instruments by commercial clients for evaluating their suppliers is a viable option. In conclusion, when fraud is suspected, it is crucial to perform sophisticated analyses to effectively assess the truthfulness of the fraudulent claims while adhering to legal boundaries. The present models might need some refinement since they need to take into consideration different causes of sample variability and the preferences of the food chain stakeholders that are interested in fish authenticity.

Acknowledgment

I express profound gratitude to my esteemed supervisor, Alessandra De Lorenzi, for her constant and resolute assistance and mentorship during the entire duration of my project. The erudition and composure of the individual in question have proven to be of incomparable worth to my educational activities, and have served as a pivotal factor in the success of this dissertation. I express deep appreciation to my friends and family members for their affection and encouragement throughout this journey. Devoid of their encouragement and inspiration, my ability to finally accomplish the mission would have been rendered impossible to achieve. Thank you.

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