UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II

Ph.D. in Food Science

Sensory methods ensuring authenticity and fostering European fish

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Thesis overview

Fish products are widely consumed around the world, as they are the main source of long-chain fatty acids, rich in protein, vitamins, and minerals and at the same time low in carbohydrates (Carlucci et al., 2015). With the globalisation of markets, fish has become a more accessible product for consumers, so thanks to lower prices, an increasing global population, better living standards and greater availability of the product, consumption has increased significantly in recent years (Tomic et al., 2016). Indeed, as reported by FAO (2014), consumption has increased by about 10 kg in 50 years, which may continue to grow (Morales & Higuchi, 2018).

The increased demand for fish products on the markets has led to an increase in fishing activities, which hurt both the marine environment and fish species (Verbeke, Vanhonacker, et al., 2007). In addition, increased demand also means more fraud, especially for less experienced consumers or for similar species, especially when sold in slices or fillets. For these reasons, many consumers are becoming sceptical about purchasing fish products and therefore perceive the presence of barriers to consumption. At the same time, companies practising environmental and social responsibility are often not fully recognized by the people. To overcome this, companies in the sector are trying to provide more and more fish-related information by labelling, such as information on traceability, sustainability, authenticity etc.

Therefore, this PhD thesis aims to assess the authenticity, traceability, and quality of Mediterranean fish products by using both analytical and affective sensory methods combined with instrumental procedures. The sensory evaluation of fish was performed with a double objective and using different procedures. On one hand, classical descriptive methods were applied to find the peculiar sensory properties confirming Mediterranean fish authenticity. Other analytical sensory methods were used to assess the quality loss of fish species during storage. On the other hand, hedonic methods were used to involve consumers and to understand their consciousness and perception of both Mediterranean and non-Mediterranean fish.

Based on the previous considerations and aims, the thesis consists of five case studies.

The first case study involved the use of descriptive analysis (QDA) to assess fish authenticity and to characterise the fish species. A part of this case study was carried out at the Department of Agricultural Science of the University of Naples Federico II, and a second part was carried out in collaboration with the NOFIMA research centre (Ås).

The second case study concerned using descriptive analysis to assess fish freshness. In particular, the Quality Index Method (QIM) was used to analyse the freshness of Mediterranean anchovies (*Engraulis enchrasicholus*). Furthermore, during this activity, an experiment was carried out to extend the short shelf-life of the anchovies through the application of a low-voltage electrostatic field (DENBA+).

The third case study focused on the development of a questionnaire sent to a group of 2000 respondents from two southern European countries, Italy and Spain, in which per capita fish consumption is high. The questionnaire aimed to analyse the consumption habits of seafood by the respondents, as well as their propensity and knowledge towards fish traceability and sustainability.

The fourth case study involved a consumer test with 105 Italian consumers. The consumer test aimed to verify the influence of external variables provided to consumers, in particular, the effect of sustainability information, on the hedonic scores for two different Mediterranean fish species.

The fifth case study was carried out in cooperation with the NOFIMA research centre (Tromsø). This activity focused on the development of a questionnaire sent to Northern European subjects to assess their propensity and knowledge for sustainability and traceability. The questionnaire was developed according to the Check All That Apply (CATA) method in which subjects had to assess perceptions of Red King Crab (*Paralithodes camtschaticus*) samples in different packaging settings.

The results collected during this thesis highlighted how sensory analysis can be a useful tool for the evaluation of parameters such as freshness, authenticity and quality, and also for assessing both consumer perceptions of seafood and their related aspects.

Sintesi tesi di dottorato

I prodotti ittici sono ampiamente consumati in tutto il mondo, sono la principale fonte di acidi grassi a lunga catena, ricchi in proteine, minerali e vitamine, e allo stesso tempo poveri in carboidrati (Carlucci et al., 2015). Con la globalizzazione dei mercati, il pesce è diventato un prodotto più accessibile per i consumatori, pertanto nel corso degli anni la domanda di prodotti ittici è notevolmente incrementata grazie alla riduzione dei prezzi, all'aumento della popolazione globale, migliori aspettative e standard di vita, e maggiore disponibilità di prodotti (Tomic et al., 2016). Come riportato da un report FAO nel 2014, i consumi pro-capite di pesce sono aumentati di circa 10 Kg in 50 anni, e ci si aspetta che questi valori possano continuare ad aumentare nel tempo (Morales & Higuchi, 2018).

La crescente domanda di prodotti ittici sul mercato comporta maggiori attività di pesca, le quali stanno risultando dannose sia per l'ambiente marino sia per le specie ittiche (Verbeke, Vanhonacker, et al., 2007). Inoltre, la crescente domanda comporta maggiori frodi, specialmente per i consumatori meno esperti, o per specie simili vendute a fette o filetti. Pertanto molti consumatori risultano scettici nei confronti delle specie acquistate, andando quindi a percepire barriere al consumo di prodotti ittici, e allo stesso tempo, le aziende che praticano la pesca in modo responsabile non sono pienamente conosciute dai consumatori. Al fine di rendere tali pratiche di pesca sostenibili e aumentare la trasparenza dei prodotti, alcune aziende forniscono maggiori informazioni ai clienti finali mediante l'uso di etichette, soprattutto orientate alla sostenibilità e alla tracciabilità.

Pertanto, tale tesi di dottorato ha avuto come obiettivo quello di verificare l'autenticità, la tracciabilità e la qualità delle specie ittiche del Mediterraneo utilizzando sia metodi sensoriali analitici sia affettivi, in combinazione con procedure strumentali. La valutazione sensoriale del pescato è stata effettuata con un duplice obiettivo e utilizzando diverse procedure. Da un lato metodi descrittivi classici sono stati impiegati al fine di trovare le caratteristiche sensoriali

peculiari per confermare l'autenticità del pescato del Mediterraneo; metodi sensoriali analitici sono stati impiegati per verificare le modifiche della qualità durante lo stoccaggio. Metodi edonici sono stati invece utilizzati al fine di coinvolgere i consumatori e di analizzare la loro consapevolezza riguardo le specie ittiche Mediterranee e non Mediterranee.

Basandosi sulle precedenti considerazioni ed obiettivi, la seguente tesi di dottorato è stata costituita da cinque casi studio.

Il primo caso studio ha riguardato l'utilizzo dell'analisi descrittiva classica (QDA) al fine di verificare l'autenticità del pescato e caratterizzare le specie ittiche. Una parte di questa attività è stata svolta presso il Dipartimento di Agraria dell'Università di Napoli Federico II, una seconda parte è stata svolta in collaborazione con il centro di ricerca norvegese NOFIMA con sede ad Ås.

Il secondo caso studio ha riguardato l'utilizzo dell'analisi descrittiva al fine di valutare la freschezza del pesce. In particolare, il Quality Index Method (QIM) è stato utilizzato per analizzare la freschezza delle alici del Mediterraneo (*Engraulis enchrasicholus*). Inoltre, questo caso studio ha anche riguardato l'utilizzo di un campo elettrostatico a basso voltaggio (DENBA+) come agente esterno per migliorare la qualità delle alici durante la conservazione refrigerata.

Il terzo caso studio ha riguardato lo sviluppo di un questionario online inviato a 2000 consumatori provenienti da due nazioni sud Europee, l'Italia e la Spagna, nazioni in cui i consumi pro-capite di prodotti ittici sono tra i più alti in Europa. I risultati del questionario hanno permesso di analizzare le abitudini di consumo dei soggetti intervistati riguardo i prodotti ittici, così come la loro propensione e conoscenza riguardo la tracciabilità e la sostenibilità ittica.

Il quarto caso studio ha riguardato un consumer test con 105 consumatori italiani. L'obiettivo di tale test è stato quello di verificare l'influenza di una variabile esterna fornita ai consumatori.

In particolare si è chiesto di valutare il gradimento edonico di specie ittiche con e senza l'informazione di un marchio di sostenibilità ittica.

Il quinto caso studio è stato svolto in collaborazione con il centro norvegese NOFIMA (Tromsø). Quest'attività ha riguardato lo sviluppo di un questionario online da inviare a consumatori Nord Europei con l'obiettivo di valutare la propensione di questi soggetti nei confronti della sostenibilità e tracciabilità ittica. Il metodo di sviluppo e compilazione del questionario è stato il Check All that Apply (CATA) su esemplari di granchio reale rosso (*Paralithodes camtschaticus*) in diversi setting di packaging.

I risultati di questa tesi di dottorato hanno evidenziato come l'analisi sensoriale possa essere un utile strumento per la valutazione di parametri del pesce come la freschezza, l'autenticità, la qualità e anche per verificare le percezioni dei consumatori riguardo il pescato e aspetti ad esso correlati.

Chapter 1

1. Introduction

1.1. Sensory analysis

Sensory analysis is a science that studies the interaction between an external stimulus (such as food, drink, sound, etc.) and the human response. The stimulus is evaluated through the use of senses such as sight, smell, taste, touch and hearing (Stone et al., 2004; Świąder & Marczewska, 2021; Torrico et al., 2023; Tuorila & Monteleone, 2009).

The birth of this science dates back to the second half of the 20th century when the expansion of the food industry and processed products began (Lawless & Heymann, 2010). In the beginning, people relied on the sensory acuity of an expert for quality products or process modifications. Over time, it was realised that it was more reliable and representative of the population to use a panel than the opinion of a single person (Lawless & Heymann, 2010; Stone et al., 2004). This science over time has become increasingly important; indeed, it is used in both academic research and industry (Świąder & Marczewska, 2021). For example, sensory analysis is used for new product or product concept development, company scale-up, process change, ingredient change, and process optimization, used as a tool to compare products between different competitors and to define the shelf life of a food product (Arnarson et al., 2011; Badwaik et al., 2014; Giménez et al., 2012; Hough & Garitta, 2012; Mosqueda-Melgar et al., 2012; Walkling-Ribeiro et al., 2010; Weinrich, 2019). Finally, considering the orientation of modern markets towards sustainable and healthy alternatives, sensory analysis can be a useful tool to understand consumers' perceptions of this category of products (Meiselman et al., 2022; Weinrich, 2019).

Sensory science has several fields of application, which can be grouped into three main categories: discriminative, descriptive, and affective tests (Lawless & Heymann, 2010; Torrico et al., 2023).

Discriminant tests are analytical tests, which are used to determine the presence of differences between two samples (Stone et al., 2004). Differences may be due to changes in formulations, or to competitors on the market. Usually, these tests are performed when the differences between the samples are slight. Discriminant tests are conducted with untrained participants, whose number depends on the type of test applied, usually between 25 and 40, selected for sensory acuity and familiarity with the test procedure (Lawless & Heymann, 2010).

Descriptive tests are analytical tests, which are used to characterise and quantify the sensory differences of a product. This type of test involves a trained panel of 8−12 assessors, familiar with the method and trained to recognise the standard references of the attributes to be recognised. To make the analysis more precise and reliable, the descriptors chosen must be appropriate to describe the product, not redundant and orthogonal. Usually, the samples are evaluated individually in a sequential monadic, balanced randomize presentation. Each descriptor is assessed using unstructured 10 cm linear scales, with only the initial and final anchorage. The assessor then freely judges by positioning along the scale (Lawless & Heymann, 2010). Sensory descriptive analysis is one of the most powerful, sophisticated and extensively used tools in sensory science (Varela & Ares, 2012). Describing the sensory characteristics of a product is a common practice in both industry and academic research, to allow informed business decisions, guide product development to match a consumers' ideal, to check the effect of ingredients or processes, for quality control purposes, to track product changes over time, and to correlate with instrumental measurements (Gracula, 1997; Moussaoui & Varela, 2010; Stone et al., 2004). Finally it is important to underline that descriptive analysis has served as a link between product characteristics and consumer reactions (Varela & Ares, 2012).

There are various ways to perform classical sensory descriptive analysis, however, the most common way to perform this kind of test is through Quantitative Descriptive Analysis (QDA). The method was created in 1974 by Stone and Sidel from the idea of creating a method that could be used to describe a wide range of products, not only foodstuffs, and that would allow

the quantification of sensory perceptions, as an alternative to existing qualitative procedures (Sidel et al., 2017). The QDA can be used to describe the product as a whole, as well as the sensations associated with the product from a visual or structural point of view up to the aftertaste (Lawless & Heymann, 2010).

As extensively described in the literature (Lawless & Heymann, 2010; Sidel et al., 2017; Stone et al., 2012), a QDA test involves a series of steps that are summarised below. Firstly the panel of judges who will conduct the analysis must be selected. As already written above, the judges have to be familiar with the method and be trained to use the rating scales, as well as being familiar with the product to be analysed. Next, the panel familiarise itself with the product or sets of products under evaluation in order to bring out the product specific list of descriptors. Next, the panel leader creates an unambiguous language of communication. This is done by selecting unique, non-redundant descriptors that well describe the products under evaluation. During this phase, the evaluation techniques for the chosen descriptors and references are also defined in order to better understand the use of the rating scale. Unstructured linear scales are used for the assessment in order to give the judge freedom during the evaluation. To better understand the scale in its entirety, references are placed at the beginning, end and sometimes in the middle of the scale (Murray et al., 2001).

Once this phase is completed, the panel of judges is trained both in the recognition of the descriptors and in the use of the rating scale. Through statistical tests, the panel leader can check the performance of the individual judge in relation to the entire panel or the entire group of judges. For the training, evaluation sessions are scheduled; when the analysis of the results shows that the panel is balanced and aligned in its evaluation, the training phase can be considered completed.

After training, the actual evaluation conducted in individual booths can start. Each session must consider replications in order to have better reproducibility of the collected results. The obtained

data would be in the form of intensity scores of all the attributes Finally, the panel leader will statistically process the collected results and define the sensory profile of the analysed products. It is common practice to look at descriptive data through sensory mapping, reducing the number of variables, and obtaining one or more biplots representing the perceptual space of interest. QDA data can be analysed with both univariate and multivariate statistical techniques. Statistical procedures such as multivariate analysis of variance, principal component analysis, factor analysis and cluster analysis have found application in the analysis of data generated by QDA-type procedures (Martens and Martens, 2001; Meullenet et al., 2007; Piggott, 1986).

The high specialization of descriptive panels allows obtaining very detailed, robust and consistent, reproducible results, stable in time and within a certain sensory space (Moussaoui & Varela, 2010). However, creating and maintaining a trained panel of judges can be very costly and time-consuming (efficient and proper panel training can take between 10 and 120 hours, depending on the complexity of the samples to be evaluated). Therefore, the economic and time demands may not be sustainable with industry needs or academic research projects. For this reason, sensory scientists have created innovative methods that are faster, more flexible and do not require extensive training. Indeed, these methods are simpler to perform and can therefore be carried out by semi-trained judges or consumers, while still providing results that are very similar to classical descriptive analysis methods (Varela & Ares, 2012).

Among the innovative methods described in this thesis is the Check All That Apply (CATA). The method makes it possible to collect information about judge perceptions of the sensory characteristics of a product under evaluation. Specifically, the judge is provided with the sample to be evaluated together with a list of terms (words or phrases); the judge must therefore select all the terms that are perceive as appropriate for the evaluated product (Varela & Ares, 2012). The list of terms may relate to sensory and non-sensory characteristics, such as emotions aroused, occasions of use, etc. The list of terms can be created from previous studies in the literature, or from preliminary group stages by a small panel of judges. As the method is

expected to be carried out by a semi-trained panel or consumers, the expected number of subjects is between 50 and 100 (Adams et al., 2007; Ares et al., 2010; Dooley et al., 2010).

The results of the CATA test are used to characterise a product or to verify differences between a set of products. The results are analysed using Cochran's Q test which is a non-parametric statistical test used in the analysis of two-way randomised block designs to check whether k treatments have identical effects, when the response variable is binary (Parente et al., 2011).

CATA questions have been used for the sensory characterization of several food products: snacks (Adams et al., 2007), strawberry cultivars (Lado et al., 2010), ice-cream (Dooley et al., 2010), milk desserts (Ares, 2010), and citrus-flavoured sodas (Plaehn, 2012). Some studies have compared the sensory maps generated by CATA questions with those provided by classic descriptive analysis (QDA) with a trained assessor panel, reporting similar results (Ares et al., 2010; Bruzzone et al., 2012; Dooley et al., 2010).

Considering these results and that checking terms from a list does not require much effort for consumers, CATA questions have been reported to be a quick, simple and easy method to gather information about consumer perception of the sensory characteristics of food products (Varela & Ares, 2012). A drawback of the CATA method is related to the position of the attributes. Indeed, as reported by Castura (2009) attributes occupying initial positions in the list receive more attention than those in the final parts. It is therefore useful that the attributes are randomised both by judge and by evaluated sample.

Innovative methods are therefore a valid, simple and quick alternative for the characterisation and description of a product, especially when there is no possibility of managing and training a trained panel of judges (Varela & Ares, 2012). It is important to emphasise that these methods cannot replace the existing classical methods, which involve a long training phase and can be used to find subtle differences in similar products. Innovative methods can be considered as

complementary methods, and can therefore be used in the preliminary characterisation stages or for the evaluation of products with substantial differences (Moussaoui & Varela, 2010).

Affective tests are used to quantify the degree of liking or disliking of a product. Unlike discriminative and descriptive tests, are hedonic tests, in which the liking degree depends on the subject's perception of the specific product. These tests are conducted with consumers, as long as the product's regulator users are being evaluated. Affective tests usually involve 75−150 consumers due to the great variability that can be present among different consumers. For this type of test, hedonic 9-point scales are usually used (Lawless & Heymann, 2010). These types of tests are carried out to test consumer response to new products, reformulations of existing products, to compare competitor products, etc.

For the proper management of a consumer test, it is important to construct an efficient experimental design, which consists of planning how many consumers to involve, standardising the sample to be tested and the tasting conditions. Depending on the type of test selected, the consumer test can be carried out either in individual sensory booths, performed at home or involve in filling out a questionnaire (Lawless & Heymann, 2010).

Regarding the questionnaire, this type of test is usually done earlier as market research to find out the opinions of a group of consumers. As extensively described by several authors (Armstrong, 1975; Furse et al., 1981; Schaefer, 1979; Sorensen, 1984; Lawless & Heymann, 2010) questionnaires require careful preparation in order to target the research aim with few but effective questions. Indeed, a questionnaire must last a maximum of 15 to 20 minutes, otherwise there is a risk of boring the interviewed consumer, who will start not paying the right attention to the questions asked, thus influencing the correct outcome of the test (Schaefer, 1979).

To improve the efficiency of a questionnaire, Lawless & Heymann (2010) recommend creating a flow chart prior to the design. The flow chart can be very detailed and include all the part of the questionnaire as well as the list of general problems.

The structure of a questionnaire always includes an introduction, where the interviewer goes to introduce the questionnaire, the objectives and to give general information for filling in (Dohrewend et al., 1968). It starts with questions of a more general nature, and then moves on to more specific questions. Often one concludes by collecting socio-demographic information, which may be useful for filtering the consumers interviewed. Generally speaking, one should try to use few but efficient questions, to have a short questionnaire (Rucker and Arbaugh, 1979), use simple language that all consumers can understand, avoid vague and ambiguous questions, as well as questions that are different but have the same meaning. Before sending the final version to consumers, it is recommended to carry out a pretest to be filled out by a small circle of consumers or experts in order to check the fluency of the test as well as to detect any errors (Schaefer 1979).

Interviews can take place in person, be self-administered on paper, conducted via a website or by telephone. Each method has advantages and disadvantages (Schaefer, 1979). Self-administration is obviously the least expensive, although being free the interviewed consumers might make more errors in attention or comprehension of the questionnaire. Telephone interviews are a reasonable compromise, but may not lend themselves to complex multi-point scales: the questions are necessarily short and direct (Groves, 1978). The in-person interview is the most flexible and the questionnaire may be complex and include a variety of scales as the interviewer and the questionnaire are both present for clarification (Boyd, 1981).

Therefore, depending on how the questionnaire is conducted, there will be a different structure to follow or a different method of data collection in order to make the research as efficient as possible. As Resurrection (1998) suggests, it is important that the rating scales always follow the same direction, from minimum to maximum, from left to right.

Considering the different fields of application of sensory analysis, the next paragraphs will be oriented towards the use of sensory analysis on fish products.

1.2.Seafood products: nutritional composition and consumption

Fish products are widely consumed all around the world thanks to their nutritional components. In particular, they are the primary dietary source of long-chain fatty acids (Omega-3), including *eicosapentaenoic* acid (EPA) and *docosahexaenoic* (DHA), but also, they are rich in proteins, minerals, and vitamins and poor in carbohydrates (Nesheim & Yaktine, 2007; Carlucci et al., 2015). Several studies report that a daily intake of 500 mg of EPA + DHA would be expected to reduce the risk of cardiovascular disease, this can be achieved by consuming at least two servings of fish per week (WHO, 2006, FAO/WHO, 2011). Moreover, fish represents a very important source of vitamin D, if compared to other usually consumed food (Graça et al., 2002; Verbeke et al., 2005). Increasing demand for fish products has been observed in the last decades due to an increase in diet-related chronic diseases, and a consumer's higher interest in healthy foods (McGuire, 2016; Willett et al., 2019). In 2014 FAO reported that global per capita fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012. It is expected that the value will reach 21.8 kg in 2050 (Morales & Higuchi, 2018). However, besides health reasons, this increase is due to the growth in world population, education level, living standards, and increased product availability (e.g: frozen, thawed, canned, and refrigerated fish) (Morales & Higuchi, 2018; Myrland et al., 2000; Tomic et al., 2016; Verbeke et al., 2007). This increasing trend in fish consumption has led to a negative ecological impact and can threaten the natural fish stocks in the sea (Carlucci et al., 2015; Verbeke, Vanhonacker, et al., 2007).

To overcome the overfishing of wild-caught species, and to meet growing population demands, at the beginning of the XXI century, aquaculture activity was proposed as a valid alternative (Cahu et al., 2004; Mitra et al., 2020). However, aquaculture is also associated with negative ecological impacts, such as the meal for the farmed species (for instance to increase weight of 1 kg of farmed fish it is necessary to 2-5 kg of fish meal; Lang et al., 2009), the modification of coastal ecosystem, the effluent production, and the impact on biodiversity (Verbeke,

Vanhonacker, et al., 2007). In addition, intensive aquaculture may involve the use of chemicals and antibiotics, which may increase drug resistance in humans (Mitra et al., 2020).

1.3.Sustainability and traceability

The negative events related to fishing have led some consumers to become more sensitive to environmental sustainability issues (Migliore et al., 2020; Pucci et al., 2020). Therefore, given the modern trends of interest in environmental sustainability (Verbeke, Vanhonacker, et al., 2007), some brands are joining sustainability programs. In this way, people can be informed about the reduced environmental impact of the food they consume (Krystallis et al., 2012; Van Loo et al., 2015). Sustainability eco-labels aim to put a tag on the label, which should be easily recognised by the subjects during purchases and thus guide their purchasing choices towards more sustainable alternatives (Annunziata et al., 2019). Figure 1 shows the main seafood sustainability labels available in European markets. Among the most well-known fish sustainability labels is the Marine Stewardship Council (MSC), an international certification label for sustainable and well-managed fisheries (Parkes et al., 2016). The sustainability objective of the MSC label is expressed in three principles: to protect fish stocks in the sea by avoiding overfishing, to use fishing techniques that do not have a negative impact on marine flora and fauna, and to properly manage fisheries (Maesano et al., 2020). The MSC label can only be attributed to capture fisheries; as far as farming is concerned, the Aquaculture Stewardship Council (ASC) label certifies farms that apply sustainable farming practices. Specifically, the ASC's aim is to protect local fauna, maintain farm water quality standards, respect harvest cycles, and follow strict guidelines for the administration of medicines and the correct formulation of farm feeds (Sherry & Koester, 2020). Another sustainability label for farmed fish products is Best Aquaculture Practices (BAP) which certifies environmentally responsible aquaculture, food safety, animal welfare, and product traceability (Roheim et al., 2012; Soley et al., 2019). Finally, another popular label is Friend of the Sea (FOS), whose main objective is the conservation of marine habitats. In contrast to the previous ones, it is applicable

for both capture fisheries and farming (Aprile & Punzo, 2022). Fishing sustainability requires that fishing practices are carried out in compliance with rules concerning quantities fished, fishing or farming techniques, respect for fishing times (growth and reproduction) and avoidance of damage to the sea (Maesano et al., 2020).

Adhering to a sustainability eco-label has a higher cost for a company, which translates into a higher cost for the consumer (Annunziata et al., 2019; Aprile & Punzo, 2022). Other authors have highlighted how the increase in the sales price may be perceived by the subjects as a barrier to consumption or may direct them to choose a cheaper but unlabelled sustainability alternative (Leek et al., 2000; Olsen, 2001; Verbeke & Vackier, 2005). Another problem related to sustainability labels is that there are often no adequate dissemination campaigns to make sustainability labels known, so many people are aware of the label, but do not know what the objectives are behind the simple label (García-Herrero et al., 2019). Although there are no adequate dissemination campaigns, other authors point out that consumers well perceive sustainability, indeed sustainable fish species are perceived positively and there is also a willingness to pay a higher price for such species (Asche et al., 2015; Mauracher et al., 2013; Roheim et al., 2011; Salladarré et al., 2016; Uchida et al., 2014).

Figure 1. Seafood sustainability ecolabel. Source google.com

The increased abundance of fish products along the supply chain can lead also to problems of authenticity, and thus, mislabelling among similar fish species could mislead less experienced

consumers (Giusti et al., 2019). For this reason, another important modern focus is on food traceability. Traceability is defined by the European Commission as "The ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution" (European Parliament and Council, 2002). Traceability is a useful tool to communicate all the information about the purchased fish product's journey from the sea to the final consumer. In addition, a traced product can guarantee higher quality, improve consumer confidence, and finally can be a useful tool for possible food-related issues (Fiorile et al., 2023; Menozzi et al., 2015; Rodriguez-Salvador & Calvo Dopico, 2023). As reported by other authors (Fiorile et al., 2023; Myae & Goddard, 2012), information about traceability is of particular interest to consumers, especially adult ones, who tend to make more conscious and conscientious purchases. As reported in other studies, the increase in food-related issues over the years has increased the need for verified and guaranteed information on food quality and safety, especially for animal and highly perishable food products (Menozzi et al., 2015).

1.4.Consumers' purchasing choices

Food-related information can influence consumers' purchasing choices, indeed during the purchase or consumption process, consumers are affected by several intrinsic factors such as the overall appearance, smell, texture, etc., and extrinsic factors such as the price, convenience, availability, ethical concerns, safety, etc. (Olsen, 2003; Thong & Olsen, 2012; Trondsen et al., 2003; Altintzoglou et al., 2011; Honkanen et al., 2005; Myrland et al., 2000; Thong & Solgaard, 2017). In addition, individual factors related to social-demographic variables play also an important role in the purchase time (Myrland et al., 2000; Olsen, 2003; Trondsen et al., 2003). As other researchers suggest, high-income and elderly consumers were found to be more likely to frequently choose fish products, if compared to young and low-income groups (Myrland et al., 2000; Olsen, 2003; Trondsen et al., 2003; Trondsen et al., 2004). Moreover, household composition plays a key role in fish consumption habits and purchase behavior: single

consumers are used to eating fish less frequently than consumers who live with other family members or partners (Thong & Solgaard, 2017). The presence of kids and teenagers in the household is detrimental to fish consumption, as they perceive the taste and presence of bones as a barrier to fish consumption (Leek et al., 2000; Olsen, 2001; Verbeke & Vackier, 2005; Trondsen et al., 2003). Gender also impacts fish consumption, in particular males eat fish products less frequently than females (Thong & Solgaard, 2017).

Regarding extrinsic factors, fish price is classified as the main cue considered during purchase, and this perception changes with the provenance country. For instance, eastern-central European countries and Baltic states feel the fish price as a strong consumption barrier; while Scandinavian countries, France and Spain also perceive the price as a consumption barrier, but they associate the high price with a quality cue, and for this reason, they are willing to pay more to obtain quality products (Brunsø et al., 2009; Vanhonacker et al., 2013). Convenience also plays a key role as an extrinsic variable in food choice, because, convenience means saving time during the purchase, storage, food preparation, and also during consumption, and the cleaning process (Gofton, 1995; Olsen et al., 2007). Although intrinsic attributes are of primary importance when purchasing a food product, consumers who are not emotionally attached to particular products may be driven in their purchases by extrinsic and ethical factors especially by brand, packaging and price (Jaeger et al., 2017; Spinelli et al., 2015). These factors generate consumer expectations of the product, which may be confirmed or disconfirmed during consumption (de Andrade et al., 2018; Deliza, 1995). Confirmation occurs if the generated expectation reflects the sensory characteristics of the product, whereas disconfirmation occurs if the generated expectation does not reflect the sensory characteristics of the product (Di Monaco et al., 2004; Murray & Delahunty, 2000). The expectations generated can be of two types: sensory or hedonic. In the first case, the consumer believes that the product possesses peculiar sensory characteristics with specific intensities, which may influence liking during consumption. In the second case, the consumer is influenced (positively or negatively) by

information related to the product (sustainability, traceability, etc.) (Caporale & Monteleone, 2001; Di Monaco et al., 2004).

1.5.Fish spoilage

Regarding the quality, fish have musculature composed of many cells joined by sheets of connective tissue anchored to the skeleton and skin. This anatomy is ideal for the bending muscle movements required for swimming in the water (Figure 2). Differences in fish muscle determine its commercial value and shelf life. In many species, muscles are white or otherwise light in colour, with alternating dark areas. The amount of dark areas depends on the type of "activity" of the species. The more active the species, the greater the amount of dark muscle present. Dark muscles are related to lipid content, in particular, the higher the lipid content, the greater the dark zones. Dark muscles (rich in lipids) give the species particular sensory characteristics, but also result in a higher rate of decay, as lipids are subject to oxidation (Hyldig et al., 2007).

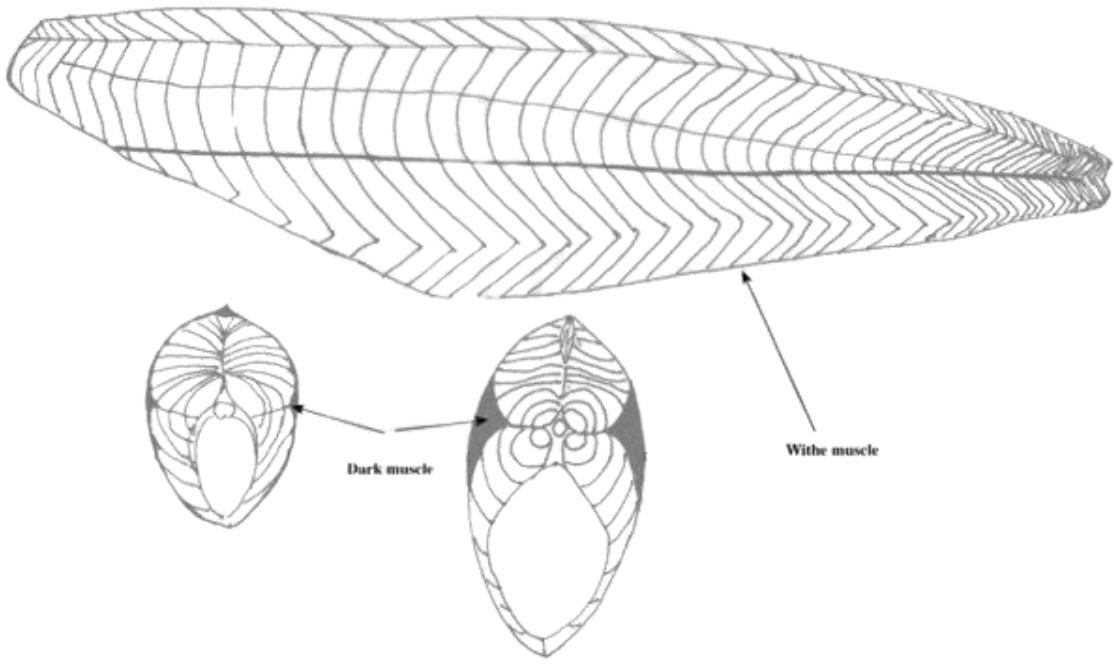

Figure 2. Muscle fish structure. Source (Hylding et al, ,2007)

Due to autolysis, that concern a series of enzymatic, chemical and microbiological reactions that occur from the post-catch phase until final consumption (Prabhakar et al., 2020; Hicks,

Pivarnik and McDermott, 2008), fish are considered perishable food products. Fish spoilage results in the formation of various degradation compounds responsible for changes in texture, odour and taste (Ghaly et al., 2010).

The high perishability is due to the fragility of fish species. Indeed, it can easily happen that fish products are compromised during fishing, transport and storage operations, due to the mismanagement of these operations which can lead to tissue damage that accelerates spoilage, as well as the failure to maintain proper refrigerated or frozen storage conditions. For these reasons, it requires accurate controls throughout the production chain, and sensory analysis is an ideal tool to carry out these controls (Hyldig et al., 2007). As reported by several authors, (Rumape et al., 2022; Panza *et al.*, 2022; Prabhakar *et al.*, 2020) a modern challenge is trying to reduce fish spoilage and increase their shelf-life. The first step is certainly to avoid mishandling practices that may lead to contamination (Kostesha et al., 2016). After that, preservation techniques can be applied to best preserve quality. Common techniques, for preserving the shelf life of fish, include temperature reduction (refrigeration and freezing). This technique does not inactivate microorganisms but slows down their metabolism, which is responsible for spoilage (Rumape et al., 2022). There must be no change in temperature during storage, and freezing, it must be done quickly to avoid the formation of large ice crystals that lead to cellular damage (Yu et al., 2018). A further preservation technique can be linked to the reduction of enzyme activity to slow down autolysis reactions. This technique, therefore, involves gutting and cleaning fish immediately after capture to remove the enzymes from the digestive tract (Ghaly et al., 2010).

Among the emerging preservation technologies, the electrostatic field has gathered increased attention because of its high efficacy in preventing food quality decay and assuring food safety (Peng et al., 2023). According to the output voltage, it is categorized as high voltage electrostatic fields (HVEF) (>2.5 kV) and low voltage electrostatic fields (LVEF) (≤2.5 kV) (Liu et al., 2023). The application of HVEF has been investigated for the preservation of tilapia,

tuna, catfish fillet, common carp, and shrimps, while as reported by Peng *et al.* (2023) the effect of LVEF was studied only for fruits and vegetables. In this context, a new technology commercially available is a device (DENBA +) which uses electromagnetic electrostatic waves generated by low-voltage static electricity to resonate with water molecules in the food cell (Yang et al., 2023). This innovative technology has the advantage of being eco-friendly for the environment, not coming into direct contact with the food and, finally, does not require much energy. Yang and colleagues (2023) tested this technology while storing strawberries, achieving better preservation of the product stored at 4 °C than the control samples. However, no work on fresh fish has been reported in the literature.

Fish landed quality is measured for years through the EU scheme. This scheme uses a three-level assessment: E, A and B. "E" stands for extra and is assigned to very fresh species, often still in rigour mortis, caught within hours of landing. "A" is assigned to fresh species, while "B" is assigned to species no longer available for human consumption. The limitation of the EU scheme is that it does not consider differences between species and can be influenced by the subjective parameters of the evaluator (Triqui & Bouchriti, 2003). Therefore, other freshness assessment schemes have been developed over time. In particular, the method assessed as most reliable for fish evaluation was the Quality Index Method (QIM). The method was developed by the Tasmanian Food Research Unit and it is currently used for about 40 species (Massa et al., 2012). The method is based on the evaluation of the parameters changing most during the fish shelf life, such as the eye, smell, gills, etc. Each attribute is assigned a demerit score ranging from 0 to 2-3 (depending on the importance of the assessed attribute) (Cardenas Bonilla, Sveinsdottir and Martinsdottir, 2007; Hyldig, Ditte and Green-Petersen, 2008). At the end of the assessment, adding up the scores of the individual attributes will result in a score, called Quality Index (QI), which will be 0 or close to 0 if the examined fish is fresh, and will deviate increasingly from 0 if the fish has deteriorated. The method is quick and easy, however, it is species-specific, so a scorecard with species-specific attributes must be developed

for each assessed fish (Massa, Manca and Yeannes, 2012; Cardenas Bonilla, Sveinsdottir and Martinsdottir, 2007; Hyldig, Ditte and Green-Petersen, 2008).

On the other hand, as far as the evaluation of cooked fish is concerned, the Torry method is the widely used one. The method involves the use of a 10-point scale developed by the Torry research station. A score of 10 is assigned to fresh species for both odour and taste, while a score of 3 is assigned to a species, not for human consumption. A consumption limit is usually used for a score of 5.5. As for the UE scheme, also this method does not consider species differences (Hyldig et al., 2007).

1.6.Thesis aim

Considering the different aspects listed in this introduction, and the versatility of sensory analysis, the aim of this PhD thesis is to use sensory analysis to assess the authenticity, quality and traceability of fish products by using both analytical and affective sensory methods combined with instrumental procedures. Classical descriptive methods were applied to find the peculiar sensory properties confirming fish authenticity. Other analytical sensory methods were used to assess the quality loss of fish species during storage. Finally, hedonic methods were used to involve consumers and to understand their consciousness and perception of both Mediterranean and non-Mediterranean fish. In particular, descriptive analysis was used with the aim of characterising and discriminating similar fish species or those with different origins, as well as assessing the freshness and quality of the fish. Physical methods were also applied to study the rapid spoilage of fresh fish. Finally, consumer knowledge on issues such as traceability and fish sustainability were investigated and how these additional variables could change consumer perceptions during purchase and consumption.

Each case study involved different fish species since there is high variability between different species and the different case studies were conducted at different times (therefore with different fish availability) and in different locations (Northern and Southern Europe).

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Chapter 3

Quality index method to evaluate fish freshness

Published paper

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Abstract

The European anchovy (*Engraulis encrasicolus*) being a species very rich in long-chain fatty acids, is subject to rapid spoilage with consequent sensory losses. Therefore, this study aimed to verify the effect of a low-voltage electrostatic field (DENBA+) on the quality of fresh anchovies in a refrigerated environment. During 9 days of storage, anchovy samples were analysed from a chemical (TVB-N and TBARS), physical (texture analysis), microbiological (microbial count) and sensory (QIM) point of view and compared to control samples (without the electrostatic field). DENBA+ technology was able to reduce of 20% the QI score increment rate concerning the control sample justified by a better quality of the samples after 7 days of storage. In addition, loads of spoiling microbes were significantly lower in DENBA+ after 9 days of storage. This non-thermal technology has shown positive effects for long periods of storage.

Keywords: fish perishability, shelf-life, fish preservation technique, trained panel, fish spoilage.

1. Introduction

The European anchovy (*Engraulis encrasicolus*) is a pelagic species that lives at depths of up to 400 metres (Renzi et al., 2019). During the summer period, it prefers surface waters, rich in mesozooplankton, while during the spawning period, it settles in lagoons, estuaries and lakes, thanks to its resistance to salinity (5 to 41 practical salinity unit (PSU)) (Borme et al., 2009). It is a species of high commercial value in Europe, both for human consumption and also as a major element in the diet of pelagic predators (Kraus et al., 2015; Renzi et al., 2019). Indeed world per capita consumption are about 1.2 kg per year of fresh product (Vázquez-Sánchez et al., 2020). Besides their taste, the wide consumption of anchovies is also due to their nutritional composition, as they are rich in polyunsaturated fatty acids (Carlucci et al., 2015; Willett et al., 2019). Being fatty species, they are very perishable. Overall fish are very perishable food products due to a series of enzymatic, chemical and microbiological reactions that occur from the post-catch phase until final consumption (Prabhakar et al., 2020; Hicks, Pivarnik and McDermott, 2008). Fish spoilage results in the formation of various degradation compounds responsible for changes in texture, odour and taste (Ghaly et al., 2010). As reported by several authors, (Rumape et al., 2022; Panza *et al.*, 2022; Prabhakar *et al.*, 2020) a modern challenge is trying to reduce fish spoilage and increase their shelf-life. The first step is certainly to avoid mishandling practices that may lead to contamination (Kostesha et al., 2016). After that, preservation techniques can be applied to best preserve quality. Common techniques, for preserving the shelf life of fish, include temperature reduction (refrigeration and freezing). This technique does not inactivate microorganisms but slows down their metabolism, which is responsible for spoilage (Rumape et al., 2022). There must be no change in temperature during storage, and freezing, must be done quickly to avoid the formation of large ice crystals that lead to cellular damage (Yu et al., 2018). A further preservation technique can be linked to the reduction of enzyme activity to slow down autolysis reactions. This technique, therefore, involves gutting and cleaning fish immediately after capture to remove the enzymes from the

digestive tract (Ghaly et al., 2010). Although washing and low temperature can help the preservation of the fish, the shelf life of the fresh anchovy is still very short (Chotimarkorn, 2014). Thus, safe and sustainable alternative treatment is required to extend the product's shelf life.

Among the emerging preservation technologies, the electrostatic field has gathered increased attention because of its high efficacy in preventing food quality decay and assuring food safety (Peng et al., 2023). According to the output voltage, it is categorized as high voltage electrostatic fields (HVEF) (>2.5 kV) and low voltage electrostatic fields (LVEF) (≤2.5 kV) (Liu et al., 2023). The application of HVEF has been investigated for the preservation of tilapia, tuna, catfish fillet, common carp, and shrimps, while as reported by Peng *et al.* (2023) the effect of LVEF was studied only for fruits and vegetables. In this context, a new technology commercially available is a device which uses electromagnetic electrostatic waves generated by low-voltage static electricity to resonate with water molecules in the food cell (Yang et al., 2023). This innovative technology has the advantage of being eco-friendly for the environment, not coming into direct contact with the food and, finally, does not require much energy. Yang and colleagues (2023) tested this technology while storing strawberries, achieving better preservation of the product stored at 4 °C than the control samples. However, no work on fresh fish has been reported in the literature.

Thus, the work aimed to study the effect of low-voltage electric field technology on the quality of fresh anchovy during refrigeration storage.

Quality was assessed using the Quality Index Method (QIM), one of the most widely used methods for assessing the freshness of raw fish as it is quick and easy to apply (Massa, Manca and Yeannes, 2012; Cardenas Bonilla, Sveinsdottir and Martinsdottir, 2007; Hyldig, Ditte and Green-Petersen, 2008; Freitas, Vaz-Pires and Câmara, 2021; Vázquez-Sánchez *et al.*, 2020) Moreover, physical, chemical and microbiological analysis were also evaluated. Basic

knowledge of quality changes of anchovy would benefit its consumption for human food as well as improve commercial practices.

2. Materials and methods

2.1. Anchovy's samples preparation and storage condition

Fresh anchovies (*Engraulis encrasicolus*), caught in October and November 2022, were bought from a fishmonger in Portici (Naples, Italy). Just after purchase the samples were placed in polystyrene boxes and covered with melted ice (fish/ice ratio: 1:2). Boxes have been perforated to allow proper leaching of fluids. The boxes were stored in two different conditions: 1. in a classic refrigerated chamber (control samples); 2. in a refrigerated chamber equipped with a generator of low voltage electric field, hereafter called DENBA+ (LVEF, 220V, 50 Hz) (DENBA+ Fresh Pro ® Technology, Tekno Fresh Italia srl, Isernia). Both the refrigeration chambers were placed at 4 ± 1 °C, and the melted ice was daily replaced. The quality of samples was studied during storage at different times using sensory, microbiological, chemical and physical analysis.

2.2.QIM evaluation

Nine assessors, with previous experiences in fish evaluation, were involved in the QIM protocol (1 male and 8 female, average age 29). The sensory scheme used for the evaluation of anchovies' freshness was the same used by Massa, Manca and Yeannes (2012), in which they used a list of ten attributes grouped into four categories concerning the general appearance, the eyes, the gills, and the consistency, for a total demerit score of 28 (Table 1). The assessors participated in three training sessions, in which they became familiar with anchovies at different storage times and with the sensory QIM scheme. Finally, freshness evaluations were carried out in three different replications. Each evaluation was performed in a single sensory booth. Assessors evaluated the anchovies from day one until day nine of shelf-life. During the evaluation, each sample was coded with a three-digit number and served to the assessor, who

did not know the actual shelf lifetime of the analysed sample. During the evaluation, the assessors had the opportunity to compare the evaluated sample with the fresh reference (anchovies at day 0). To avoid problems related to intra-sample variability, five samples of anchovies were served to each taster in each session. The scores for all the evaluated parameters were added to obtain the total quality index (QI), which could be 0 or close to 0 if the examined fish is fresh and deviate increasingly from 0 if the fish has spoiled.

Table 1. QIM scheme

Category	Attribute	Description	Score
General appearance	Surface appearance	Bright, dark blue iridescence in dorsal and silver in the ventral surface	0
		Less bright with loss of iridescence	1
		Slightly dull, not bright	2
		Dull, the skin is removed easily from the flesh	3
	Skin	Complete	0
		Slightly broken	1
		Torn and damaged mainly in the abdomen	2
	Operculum	Silver bright, bloodiness no visible	0
		Less bright, slight bloodiness	1
		Some bloodiness (<50%)	2
		Very bloodiness (>50%)	3
Eyes	Cornea	Clear, transparent	0
		Slightly cloudy	1
		Cloudy, opaque	2
	Pupil	Bright black	0
		Dull black	1
		Grey	2
		Grey and distorted	3
	Shape	Convex	0
		Flat	1
		Slightly sunken	2
		Sunken	3
Gills	Colour	Bright red	0
		Dull-red	1
		Pink/light brown	2
		Discoloured grey-brown	3
	Smell	Fresh, seaweedy	0
		Neutral	1
		Rancid, fishy	2
		Off-odour, putrid	3
Consistency	Texture	Rigid (Rigor)	0
		Firm and elastic	1
		Slightly soft, less elastic	2
		Very soft	3

Abdomen	Intact	0
	Soft	1
	Slightly burst	2
	Burst	3
Quality index (QI)		0-28

2.3.Microbiological analysis

Control and DENBA+ samples were analysed in triplicate at time 0 and after 1, 2, 3, 5 and 9 days of storage. About 25 g of anchovies were immersed in a ratio of 1:10 with quarter-strength Ringer's solution (Oxoid, Basingstoke, UK) and manually shaken to detach microbial cells from the surface, avoiding the disruption of the tissues. Serial dilutions were performed and plated on PCA (Plate Count Agar, Oxoid), VRBGA (Violet Red Bile Glucose Agar, Oxoid), *Pseudomonas* agar base (PSA) supplemented with the CFC supplement (Oxoid) and STAA agar (Streptomycin Thallous Acetate Actidione, Oxoid) with the addition of STAA selective supplement (Oxoid).

All the plates were incubated aerobically for 48 hours at 37 °C (VRBGA, for *Enterobacteriaceae*), at 20 °C (PSA, STAA and PCA, for *Pseudomonas* spp., *Brochothrix thermosphacta and* the total mesophilic psychrophilic populations), and 30 °C (PCA, for the total mesophilic populations).

2.4.Chemical analysis

For chemical analysis, control and DENBA+ treated anchovies (100 g each) were taken in triplicate during the storage time (0, 1, 2, 3, 5 and 9 days). Samples were manually decapitated, gutted, cleaned, and then homogenized to a soft paste by using a three-blade hand blender (Kenwood, Hachiōji, Japan). The obtained paste was immediately used for the estimation of total volatile basic nitrogen (TVB−N) and thiobarbituric acid reactive substances (TBARS).

2.4.1. TVB−N

TVB−N was determined following the protocol of Taher et al. (2013) with some modifications. Twenty-five grams of homogenized anchovy paste and 50 mL of 7.5% TCA (trichloroacetic acid, w/v) (Carlo Erba, Italy) were placed in a 100 mL beaker and then mixed with the three-blade hand blender (Kenwood, Hachiōji, Japan). The sample was stored for 10 min at 4 °C and then transferred in a centrifuge tube and centrifuged for 5 min at 20000×g at 4 °C. Twenty-five mL of the supernatant was poured into a glass distillation tube and added with 10 mL of 10 % NaOH (Carlo Erba, Italy). The volatile nitrogen was steam distilled into 60 mL of 4% Boric Acid (Carlo Erba, Italy) using the K-350 Distillation Unit (BÜCHI, Switzerland); distillation continued until a final volume of 100 ml was obtained in the flask (40 mL of distillate). The distilled nitrogen was titrated with 0.01 M HCl (Carlo Erba, Italy) until pH 4.29 (Five Easy F20 pHmeter, Mettler Toledo). Twenty-five mL of 7.5% TCA (Carlo Erba, Italy) were distilled and titrated as the blank sample.

The TVB−N content was expresses as mg of nitrogen per 100 g of sample and calculated following equation 1:

$$\text{TVB} - \text{N} = \frac{(V_s - V_0) \times 14.007 \times a}{m} \times \frac{V_{extr}}{V_{dist}} \times 100$$

Where,

V_s and V_0 are the volume (mL) of M HCl used to titrate the sample and the blank respectively,

14.007 is the molar mass of Nitrogen (g mol^{-1})

a is the concentration of titrant (mol L^{-1})

m is the weight of the sample (in grams),

V_{extr} and V_{dist} are the total volume of extract and the volume used for the distillation of nitrogen (mL), respectively.

Samples were analysed in triplicate (n=3).

2.4.2. T BARS

For TBARS determination a spectrophotometric assay was used according to Maraschiello et al. (1999) with some modifications. One g of freshly homogenized anchovy paste was mixed with 10 mL of distilled water and vortexed for 1 minute. Then, 2.5 mL of 25% TCA (w/v) was added and the sample was stored for 10 min at 4 °C and finally centrifuged for 5 min at 20000×g at 4 °C. Aliquot of 1.2 mL from the supernatant was mixed with 0.5 mL of daily prepared 0.6 % TBA (thiobarbituric acid, w/v) and incubated for 30 min at 70 °C. After heating the sample was allowed to cool to room temperature and the absorbance at 532 nm was measured through a UV/VIS spectrophotometer (V-730, Jasco, Sud Africa), using a solution containing 5% TCA and 0.5 mL of TBA (0.6%) as blank. The concentration of T BARS, expressed as mg of MDAequiv/kg of fresh sample, was extrapolated from a calibration curve obtained with Standard MDA based on acidic hydrolysis of TEP (1,1,3,3-Tetraethoxypropane) (Maraschiello et al., 1999). Samples were analysed in triplicate (n=3).

2.5.Texture analysis

Control and DENBA+ samples were analysed in triplicate every day for 9 days of storage. The whole sample was subjected to a simple compression test using a texturometer (TSM-Pro, Food Technology Corporation, Slinfold, England) equipped with a 0.5 cm diameter cylindrical probe and with loading-bearing cells (50N). Tests were run with a crosshead speed of 120 mm/min, with a trigger force of 0.08N. The data was collected using Texture Lab Pro software, and the maximum force at the yield point (hardness, N) was calculated.

2.6.Data analysis

Linear regression was used to describe the increment of QI during storage time for both samples. Analysis of variance (two-way ANOVA and Duncan test, $p \leq 0.05$) was used to determine the differences between the parameters connected to fish freshness along the storage time. In particular, the test was used to analyse the differences between treatments (control and

DENBA+) and storage time (days). All the data are reported as mean ± SD (standard deviation). The confidence level was 95% in every statistical test used. The analyses were done by using SPSS statistical software (SPSS vers. 28 Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Quality index method

Fresh anchovies (time 0) are characterised by a bright livery with blue veins, a silvery operculum, a clear, transparent cornea, a bright black pupil, and a perfectly convex eye shape. The gills are bright red, the abdomen intact, and a rigid texture (rigor mortis) (Pons-Sánchez-Cascado et al., 2006). During ice storage, anchovies change due to chemical and microbiological reactions, as well as surface bacteria and digestive enzymes, which lead to quality loss (Prabhakar et al., 2019). The surface loses its brightness, becomes dull and the skin tends to tear. The operculum fills with blood, and the eye loses its shape and tends to disappear. The gills discolour, the abdomen bursts and the texture becomes soft (Massa, Manca and Yeannes, 2012; Pons-Sánchez-Cascado *et al.*, 2006). The parameters evaluated during the study showed a linear upward trend over time for both treatments . Figure 1 shows the QIM graphs of anchovies stored in the presence of DENBA+ and control, respectively. The scores increase for both trials during ice storage with statistically significant differences (two-ways ANOVA, Duncan test $p \leq 0.05$). The maximum score achieved by the anchovies was 20 in the control samples and 15 in the trial with DENBA+. As can be noted in Figure 1, the scores of the control anchovies, linearly increase until day 9 of ice storage, whereas the scores of the anchovies stored in the refrigeration chamber equipped with DENBA+ increase up to an equilibrium score reached after 6 days of storage. Describing the QI increment by using linear regression (R^2=0.97 for the control sample, R^2=0.96 for SEF samples), as required by the QI method, results clearly showed that the DENBA+ technology was able to reduce the QI score increment rate (slope = 2.0 day^{-1}) compared to the control sample (slope = 2.5 day^{-2}). The scores obtained by control

anchovies are consistent with other studies on anchovies' QIM (Massa, Manca and Yeannes, 2012; Pons-Sánchez-Cascado *et al.*, 2006). In particular, Massa et al. (2012) obtained a QIM curve with an R^2 of 0.98 and Pons-Sánchez-Cascado *et al.*, (2006) with an R^2 of 0.92. The differences that can be observed with the other authors may be due to sample variability, e.g. the period of conducting the analysis, which may lead to differences in anchovies' size or biological and trophic differences of samples. The reduction in the QI score of anchovies stored in the refrigeration chamber with DENBA+ may be because of the electrostatic field, which impacts on the cellular structure, preserving its rupture and liquid loss. In this way, the characteristics of the anchovies are improved during storage on ice. Our results are consistent with Xanthakis *et al.*, (2013) and Dalvi-Isfahan, Hamdami and Le-Bail, (2016), by applying a static electric field to pork and lamb meat, they obtain a product with less damage to the internal structure, thus achieving better preservation than during classic refrigerated or frozen storage.

By comparing the two treatments, statistically significant differences were found ($p \leq 0.05$). There is a first difference after three days of storage. But the differences are evident from day 7 onwards. Thus, since the scores of the two treatments are similar for short storage time, the preservative effect of the static electric field produced by DENBA+ is more evident for a longer shelf-life.

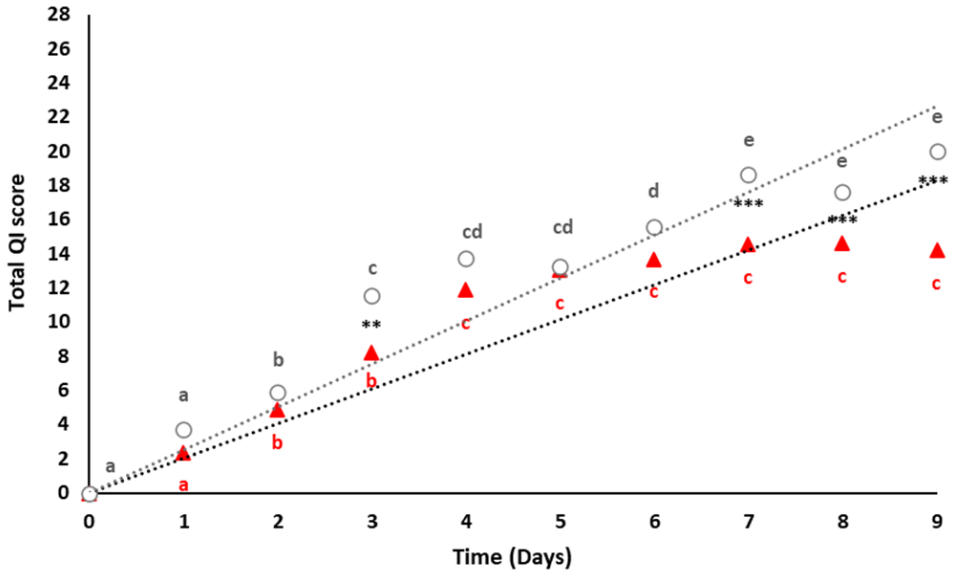

Figure 1. QIM results of anchovies stored with DENBA+ (▲ R2= 0.9596) and control (● R2= 0.9747). Different letters correspond to statistically significant differences among the time (Duncan test p≤0.05). Asterisks indicate significant differences between treatments (* p<0,05; ** p<0,01; *** p<0,001).

3.2.Microbiological analysis

Initial psychrophilic and mesophilic viable counts of anchovy muscle at baseline were 4.2±0.7 and 3.8±0.17 log CFU/g, while *Pseudomonas* spp., *B. thermosphacta* and *Enterobacteriaceae* were 3.9±0.6, 1.9±0.4 and 1.6±0.3 CFU/g, respectively. By applying two-way ANOVA (Duncan test $p \leq 0.05$) analysis, statistically significant differences in microbial growth can be verified for the samples analysed along the nine days of storage ($p \leq 0.05$). Only the *Enterobacteriaceae* count in the control samples showed no statistically significant differences ($p \geq 0.05$). No significant differences in TAP and TAM between the DENBA+ and the control groups were observed during the shelf-life (Table 2). However, lower counts were observed in DENBA+ at 9 days of storage (although significant only for TAP). Starting from 3 days of storage up to 9 days, *Pseudomonas* spp. was lower in DENBA+, reaching 7.5±0.8 and 8.3±0.5 CFU/g in DENBA+ and control anchovies, respectively (Table 2). Consistently, also *B. thermosphacta* was about 1 log lower in DENBA+ at the end of storage.

Table 2. Log CFU/g of the total aerobic mesophilic population (TAM), total aerobic psychrophilic population (TAP), *Enterobacteriaceae*, *Pseudomonas* spp. and *Brochothrix thermosphacta*. The table shows the average Log CFU/g values (± SD) of three replicates.

	TAM		TAP		*Enterobacteriaceae*		*Pseudomonas* spp.		*B. thermosphacta*	
	CTRL	DENBA +	CTRL	DENBA +	CTRL	DENBA +	CTRL	DENBA +	CTRL	DENBA +
Day 0	3.8 ± 0.7^{a}	3.8 ± 0.7^{a}	4.2 ± 0.7^{a}	4.2 ± 0.7^{a}	1.6 ± 0.3	1.6 ± 0.3^{a}	3.9 ± 0.6^{a}	3.9 ± 0.6^{a}	1.9 ± 0.4^{a}	1.9 ± 0.4^{a}
Day 1	3.2 ± 0.8^{a}	3.0 ± 0.6^{a}	4.1 ± 1.2^{a}	3.5 ± 0.1^{a}	2.2 ± 0.5	2.0 ± 0.2^{b}	3.5 ± 0.9^{a}	3.9 ± 0.2^{a}	2.8 ± 0.1^{ab}	2.6 ± 0.9^{ab}
Day 2	3.3 ± 0.4^{a}	3.8 ± 0.1^{a}	4.0 ± 0.4^{a}	4.3 ± 0.4^{b}	1.0 ± 0.8	1.0 ± 0.8^{a}	3.6 ± 0.4^{a}	4.0 ± 0.5^{a}	2.0 ± 0.2^{a}	3.4 ± 0.1^{b}*
Day 3	4.1 ± 0.4^{ab}	4.3 ± 0.4^{ab}	4.6 ± 0.8^{a}	4.8 ± 0.1^{b}	2.2 ± 0.1	3.1 ± 0.1^{c}	4.2 ± 0.2^{ab}	3.8 ± 0.1^{a}*	3.0 ± 0.8^{ab}	2.8 ± 0.1^{ab}
Day 5	4.6 ± 0.7^{b}	5.7 ± 0.1^{bc}	5.0 ± 0.3^{a}	6.2 ± 0.5^{c}*	1.8 ± 1.3	1.9 ± 0.5^{b}	4.9 ± 0.5^{b}	6.4 ± 0.6^{b}*	4.1 ± 1.1^{b}	2.0 ± 1.1^{a}

Day 9	7.2 ± 0.5^{c}	6.0 ± 1.7^{c}	7.9 ± 0.2^{b}	6.7 ± 0.3^{c}*	3.8 ± 0.2	3.1 ± 0.3^{c}*	8.3 ± 0.5^{c}	7.5 ± 0.8^{b}*	6.5 ± 0.6^{c}	5.6 ± 0.4^{c}*
p-value	0.001	0.05	0.001	0.001	0.1	0.001	0.001	0.001	0.001	0.001

For each column, different letters correspond to statistically significant differences (Duncan test $p \leq 0.05$). For each row, asterisks indicate significant differences between control and DENBA+ samples for each tested microorganism (* p<0,05; ** p<0,01; *** p<0,001).

3.3.Evaluation of chemical indices and microbial counts of fresh CTRL and LVEF treated anchovies' samples during the shelf life

As muscle foods, fish composed of water, proteins and lipids are subjected to rapid spoilage leading to unwanted changes in appearance, texture, taste and odour, which reduce its quality (Tavares et al., 2021). The loss of freshness and safety of muscle foods is often evaluated through the determination of chemical compounds used as biomarkers such as TVB−N and TBARS. The degradation of proteins and amines is the main responsible source of TVB−N through the formation of trimethylamine-N-oxide, trimethylamine, and dimethylamine (Bekhit et al., 2021).

The content of TVB−N was monitored during storage in anchovies refrigerated at 4±1 °C in control and under DENBA+ treatment. As shown in Figure 2A, up to day 7 of refrigerated storage, the control and DENBA+ samples did not show significant differences in TVB−N levels, which remained almost constant in a range of 6−10 mg/100 g of fresh sample, with a slight decreasing trend attributable to the fact that amines are volatile and the samples were refrigerated in the absence of packaging. An evident difference in TVB−N values was observed only on the 9th day of refrigeration when its content increased significantly compared to the previous days (Figure 2A). In particular, for the control sample, the estimated amount of TVB−N was 20.8±1.1 mg/100 g, while the DENBA+ sample settled at 14.1 ± 0.4 mg/100 g. By applying the analysis of variance (two-way ANOVA, Duncan test $p \leq 0.05$), statistically significant differences were found between the TVB-N scores during the nine days of storage ($p \leq 0.05$).

EC Regulation 2074/05 has defined guidelines for the freshness of unprocessed fish products by establishing TBV-N limit values ranging from 25 to 60 mg/100 g, depending on the fish species and final use, beyond which the products are not suitable for consumption. Unfortunately, the *Engraulis encrasicolus* species is not included in this regulation.

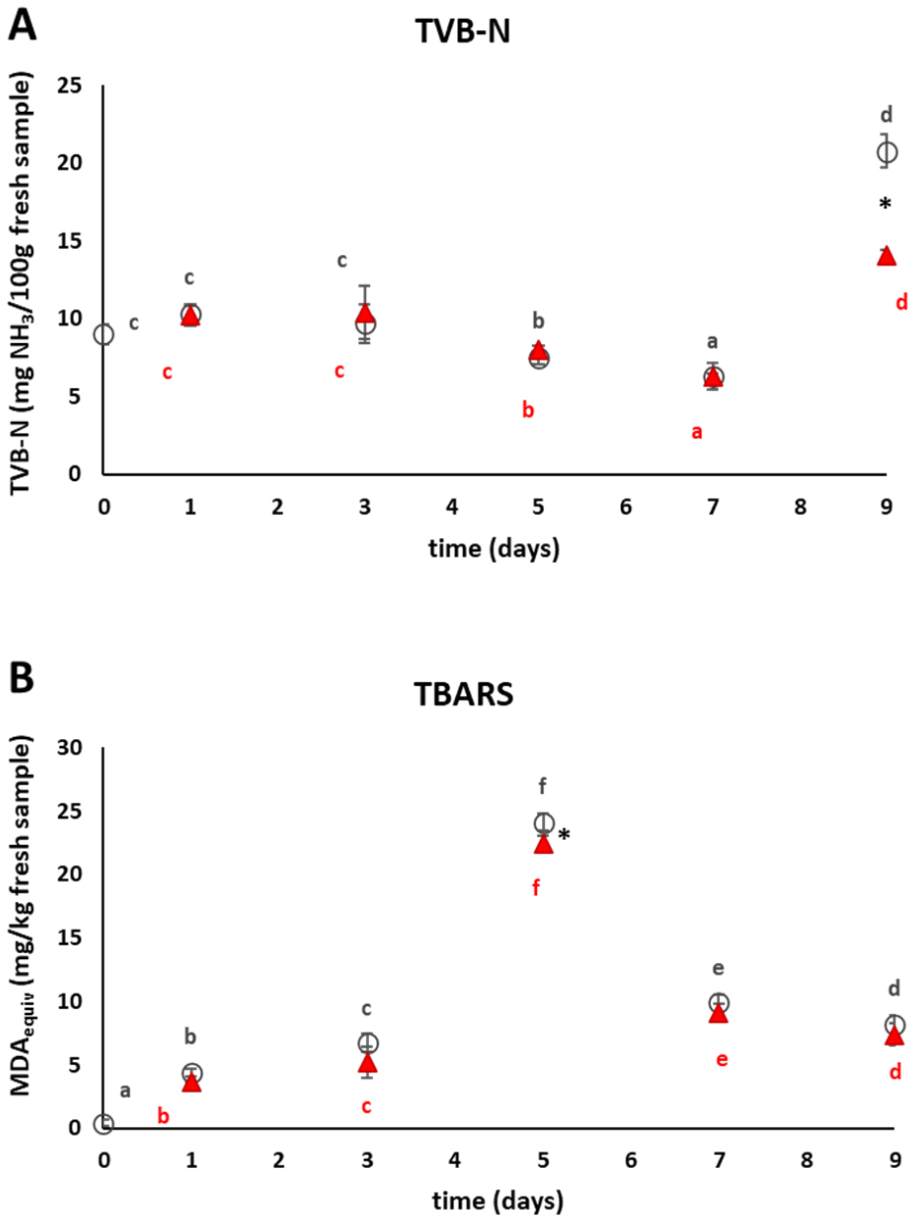

Figure 2. TVB−N (A) and TBARS (B) values, determined during storage of anchovies at 4 ± 1 °C in a classic refrigerated chamber (● CTRL) or in a refrigerated chamber equipped with a generator of low voltage electric field (▲ DENBA +). Different letters correspond to statistically significant differences among the time (Duncan test p≤0.05). Asterisks indicate significant differences between treatments (* p<0,05; ** p<0,01; *** p<0,001).

By combining the evaluation of the sensory parameters with the corresponding TBV−N values it is possible to identify guide values for the evaluation of freshness. In detail, in the fresh product, the TVB−N values do not exceed 20 mg/100 g, while from 20 to 30 mg/100 g the product is still sensory acceptable; above 30 mg/100 g the alteration phenomena begin to become evident. Based on this criterion, it is possible to state that in this study the DENBA+ samples even on the ninth day could fall into the fresh fish category, while the control samples reached the lower threshold of the acceptability range.

However, in both samples, the TVB−N content increased significantly compared to the previous days (Figure 2A). From the literature it is known that the content of TVB−N in fish products increases when the availability of glucose decreases; this promotes protein degradation by microorganisms, which use amino acids as an alternative energy source. From the catabolism of amino acids, biogenic amines are obtained (Bekhit et al., 2021). It can, therefore, be hypothesized that between the 7th and 9th day, available glucose becomes scarce to the point of inducing microbial populations to reprogram their metabolism on nitrogenous compounds, which explains the increase in TVB−N values. However, it is important to underline that treatment with DENBA+ produces the effect of significantly reducing the increase in volatile amines compared to the control, suggesting a lower metabolic activity of amine-producing microorganisms. Although no effect of DENBA+ was observed on *Enterobacteriaceae* counts during the storage, all the populations monitored (including *Enterobacteriaceae*) showed lower counts in DENBA+ treated anchovies at 9 days of storage (Table 2). *Enterobacteriaceae* are well known for proteolytic activity and are considered as the primary producers of amines in fish (Arulkumar et al., 2023).

Another important qualitative aspect in evaluating the degree of freshness of fish products is the state of lipid oxidation. In fact, in fatty species, the processes of protein degradation also overlap with the phenomena of rancidity affecting the lipids, mostly unsaturated. Lipid oxidation is among the major causes of deterioration of foods containing polyunsaturated fatty

acids, which are first oxidized to form hydroperoxides, which can be further decomposed into secondary products. Malondialdehyde is one of the main aldehydes that are generated by the secondary oxidation of lipids, for this reason, it is used as a marker of the aforementioned phenomena and it can be followed thanks to its ability to react with thiobarbituric acid. In this study, as already observed for volatile amines, the TBARS values are also practically comparable for the control and DENBA+ samples during the storage period, except on the 5th day where a difference, albeit slight, but significant ($p < 0.05$) is observed (Figure 2B). As expected, TBARS levels increased over time, going from values of 0.45 ± 0.22 mg/kg of fresh sample determined at time zero to 24.1 ± 0.7 and 22.5 ± 0.5 for the control and DENBA+ samples respectively on the fifth day of storage, which represent the highest values recorded during the experiment. In the following days, the values decreased and almost reached the levels of the first days of storage (Figure 2B). By applying the analysis of variance (two-ways ANOVA, Duncan test $p \leq 0.05$), statistically significant differences were found between the TBARS scores during the nine days of storage ($p \leq 0.05$).

These results are consistent with the existent literature, indeed it is reported that TBARS values should increase in the early stages of storage and then undergo a decrease as storage continues. This phenomenon could be due to a complexation that occurs between the proteins present in fish muscle, in particular myosin and aldehydes, throughout the storage period (Karaçam et al., 2002).

There are no legislative limits regarding the MDA content in fish products but, in the bibliography, the threshold of 0.5 mg MDA_{equiv}/kg of fresh product is considered as a limit to indicate the acceptability of the fresh product, in terms of development of rancidity and off flavours. Values between 0.5 and 1.5 mg/kg indicate a product that is still acceptable; above this value, the product is considered rancid (Ke et al., 1984).

In the experiments reported in this study, the sample at time zero could fall into the fresh category even if the TBARS values were already close to the threshold of 0.5 mg/kg. At all other times, the TBARS values were also higher than the limit of 1.5 mg, considered acceptable, and the treatment applied did not prove effective in counteracting the lipid oxidation of the anchovies.

3.4.Texture measurements

Total hardness is an important parameter for the commercial value of meat products (Lung et al., 2022; Xie et al., 2021). Immediately after capture, the anchovies are very hard as they are in rigor mortis, after which, with the onset of enzymatic autolysis, the tissues start to soften (Massa et al., 2012). Table 3 shows the hardness results of anchovy samples stored with and without DENBA+. As confirmed above, hardness decreases during storage of anchovies regardless of the treatment applied. Applying the two-way ANOVA (Duncan test $p \leq 0.05$) only the samples treated with the DENBA+ electrostatic field showed statistically significant differences in hardness reduction trend along the storage time ($p \leq 0.05$). The static electric field seems to reduce the values of total hardness, however, the statistical test showed that there were no statistically significant differences between the two treatments ($p \geq 0.05$). The results are consistent with Hu and colleagues (2021), who compared the quality of thawed pork with and without a low-voltage electrostatic field (DENBA) and showed that the treated meat had a lower hardness when subjected to texture analysis. A similar effect was confirmed by Dalvi-Isfahan et al. (2016) and Lung et al. (2022), which showed lower hardness for lamb and duck samples frozen and thawed with the application of an electrostatic field compared to control samples. This effect may be due to the electrostatic field, which reduces the impact of cold on muscle tissue and also has a better water-holding capacity (Hu et al., 2021; Lung et al., 2022).

Table 3. Texture analysis results

Time (Days)	DENBA+	Control	p-value
1	4.7±0.4^{bc}	5.9±1.4	0.154

2	4.4±1.3^{abc}	6.0±2.4	0.165
3	5.6±0.3^{c}	5.1±1.0	0.234
4	5.6±1.1^{c}	6.2±0.4	0.369
5	3.9±0.8^{ab}	5.2±0.7	0.056
6	3.7±1.3^{ab}	5.0±0.9	0.262
7	4.4±1.3^{bc}	5.4±1.7	0.366
8	3.2±0.7^{ab}	4.3±1.3	0.171
9	2.9±1.0^{a}	3.7±1.2	0.357
p-value	0.001	0.107	

For each column, different letters correspond to statistically significant differences (Duncan test $p \leq 0.05$).

4. Conclusion

Although anchovies are very perishable fish species, as they are small in size and very rich in fat, the application of the static electric field DENBA+ had a significant impact during the storage at 4±1 °C on the anchovy quality. In particular, DENBA+ showed improvements from a microbiological, chemical, physical and sensorial point of view, but this effect was only evident after at least 7 days of storage in a refrigerated environment. Further studies could concern the application of the DENBA+ electrostatic field with storage temperatures below refrigeration, as well as applying this technology to other fish species, of different sizes. Moreover, other instrumental parameters, such as colour and pH could be measured to better understand the effect of this preservative non-thermal technology.

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Chapter 4

Consumers' awareness of fish traceability and sustainability

Published paper

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Abstract

Fish products are widely consumed in different European countries for their nutritional composition, such as their high protein content, omega-3 fatty acids, minerals, vitamins, and low carbohydrates. Therefore, fishing provides important income and commercial opportunities in different Mediterranean coastal countries. As the increased consumption of fish products is leading to negative ecological impacts on marine flora and fauna, sustainability labels are increasingly emerging. Furthermore, to increase transparency in the fisheries sector and increase consumer confidence when purchasing, fish traceability is becoming increasingly important. Therefore, this study aimed to analyse the importance of fish traceability and the knowledge of some fish sustainability labels in two European coastal countries, Italy and Spain. The investigation was carried out through an online questionnaire filled out by 1913 consumers in Italy and Spain. The main results show that receiving traceability information was mainly important for the Italian population, while although fish sustainability is increasingly important, respondents did not demonstrate that they frequently buy fish products with sustainability

labels. The study also highlighted how the main characteristics of the respondents may influence their habits and perceptions regarding the issues.

Keywords: online questionnaire, food sustainability labels, consumer confidence, so-cial-demographic influence

1. Introduction

Fish products are widely consumed all around the world thanks to their nutritional composition. In particular, they are the primary dietary source of long-chain fatty acids (Omega-3), including *eicosapentaenoic* acid (EPA) and *docosahexaenoic* (DHA), but also, they are also rich in proteins, minerals, and vitamins and poor in carbohydrates (Carlucci et al., 2015; Nesheim & Yaktine, 2007). Over the years, the consumption of fish products increased, indeed as FAO reported in 2014 (FAO, 2014), global per capita fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012. It is expected that the value can reach 21.8 kg in 2050 (Morales & Higuchi, 2018). The increasing demand for fish products is due to several reasons including: an increase in diet-related chronic diseases, and a consumer's higher interest in healthy foods (McGuire, 2016; Willett et al., 2019); the globalization of the fish trade which reduced commercial costs and made fish cheaper and more abundant; the growth in world population, the increasing in education level, living standards, and increasing product availability (e.g.: frozen, thawed, canned, and refrigerated fish) (Morales & Higuchi, 2018; Myrland et al., 2000; Tomic et al., 2016; Verbeke et al., 2007). The increase in fish consumption, and consequently the increase in fishing activities, is becoming environmentally unsustainable and can threaten natural fish stocks in the sea (Carlucci et al., 2015; Verbeke et al., 2007). Therefore, given the modern trends of interest in environmental sustainability (Pucci et al., 2020), some brands are joining in sustainability programs. In this way, people can be informed about the reduced environmental impact of the food they consume (Krystallis et al., 2012; Van Loo et al., 2015). Sustainability eco-labels aim to put a tag on the label, which should be easily recognised by the subjects during purchases, and thus guide their purchasing choices towards more sustainable alternatives (Annunziata et al., 2019). Among the most well-known fish sustainability labels is the Marine Stewardship Council (MSC), an international certification label for sustainable and well-managed fisheries (Parkes et al., 2016). The sustainability objective of the MSC label is expressed in three principles: to protect fish stocks in the sea by

avoiding overfishing, to use fishing techniques that do not have a negative impact on marine flora and fauna, and to properly manage fisheries (Maesano et al., 2020). The MSC label can only be attributed to capture fisheries; as far as farming is concerned, the Aquaculture Stewardship Council (ASC) label certifies farms that apply sustainable farming practices. Specifically, ASC's objective is to protect local fauna, maintain farm water quality standards, respect harvest cycles, follow strict guidelines for the administration of medicines and the correct formulation of farm feeds (Sherry & Koester, 2020). Another sustainability label for farmed fish products is Best Aquaculture Practices (BAP). BAP certifies environmentally responsible aquaculture, food safety, animal welfare and product traceability (Roheim et al., 2011; Soley et al., 2019). Finally, another popular label is Friend Of the Sea (FOS), whose main objective is the conservation of marine habitats. In contrast to the previous ones, it is applicable for both capture fisheries and farming (Galati et al., 2015).

Adhering to a sustainability eco-label has a higher cost for a company, which translates into a higher cost for the consumer (Annunziata et al., 2019; Aprile & Punzo, 2022). Other authors have highlighted how the increase in the sales price may be perceived by the subjects as a barrier to consumption or may direct them to choose a cheaper but unlabelled sustainability alternative (Leek et al., 2000; Olsen, 2001; Verbeke & Vackier, 2005). A further problem related to sustainability labels is that there are often no adequate dissemination campaigns to make sustainability labels known, so many people are aware of the label, but do not know what the objectives are behind the simple label (García-Herrero et al., 2019). Although there are no adequate dissemination campaigns, other authors point out that consumers well perceive sustainability, indeed sustainable fish species are perceived positively and there is also a willingness to pay a higher price for such species (Asche et al., 2015; Mauracher et al., 2013; Roheim et al., 2011; Salladarré et al., 2016; Uchida et al., 2014).

Moreover, the increased abundance of fish products along the supply chain can lead to problems of authenticity, and thus fraud between similar fish species for less experienced consumers

could happen (Giusti et al., 2019). For this reason, another important modern focus is on food traceability. Traceability is defined by the European Commission as "The ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution" (European Parliament and Council, 2002). Traceability is a useful tool to communicate all the information that the purchased fish product has made from the sea to the final consumer. In addition, a traced product can guarantee higher quality, can improve consumer confidence, and finally can be a useful tool for possible food-related issues (Menozzi et al., 2015; Rodriguez-Salvador & Calvo Dopico, 2023). As reported in other studies, the increase in food-related issues over the years has increased the need for verified and guaranteed information on food quality and safety, especially for animal and highly perishable food products (Menozzi et al., 2015).

Given the current state of fisheries in the Mediterranean area and the needs of consumers, this study aims to learn about the importance given by consumers to the traceability of purchased fish species and knowledge of fish sustainability labels. The study involved two coastal European countries: Italy and Spain, to which consumers were sent a questionnaire to fill out online. The choice of these two countries is because they are both coastal countries whose economies are based on fishing. Moreover, coastal countries have the highest consumption frequency of fish products, as previously reported in 2019 (EUMOFA, 2020), per capita seafood consumption in Italy and Spain was approximately 31.21 and 46.02 kg per year, respectively. Abundant consumption may be due to the strategic location that allows them large supplies of such products and culinary traditions (Welch et al., 2002).

Since traceability is linked to the authenticity and freshness of fish species (Carvalho et al., 2015; Helyar et al., 2014), while fish sustainability labels only concern processed products (Maesano et al., 2020), and since Italy and Spain are coastal countries whose fish

consumption habits are more oriented towards fresh and local products (Welch et al., 2002), this study is based on three research questions:

RQ1. Do Italian and Spanish consumers consider it is important to have information on the traceability of the fish species they buy?

RQ2. Are Italian and Spanish consumers familiar with the most well-known sustainability labels on the market?

RQ3. Can interest in fish traceability and sustainability be influenced by individual consumer characteristics?

The investigation of the above research question will be helpful to identify the consumers' characteristics useful to develop effective market strategies oriented toward traceable and sustainable food choices.

2. Materials and Methods

2.1. Research Overview

The online questionnaire was first developed in the Italian language by the Department of Agricultural Sciences of the University of Naples, Federico II, then it was translated into Spanish by Packaging, Transport & Logistics Research Centre (ITENE). The pre-tested questionnaire was administered in May and June 2021 to respondents by two market research agencies based in Italy and Spain, respectively. The online questionnaire was sent to 2003 consumers (1003 in Italy and 1000 in Spain), but only 1913 answers were considered (961 in Italy and 952 in Spain) because some of the respondents were not compatible with the objectives of the research (different diet habits, no fish liking, no fish consumers). In order to obtain a representative sample of both the Italian and Spanish population, the balancing requirements for participation requested from the market research agencies were: age range (between 18 and 70 years old), gender (male, female), geographical location (coastal, inland city), number of inhabitants per population centre (up to 5 thousand, between 5 and 20 thousand,

between 20 and 100 thousand, over 100 thousand inhabitants), educational qualification (middle school diploma, secondary school diploma, university degree, post-graduate formation). The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of the University of Naples, Federico II. Participants signed written informed consent in double copy. The privacy rights of human subjects were always observed, and names and surnames were not collected.

2.2. Online questionnaire

The online questionnaire consisted of 10 questions, and it was subdivided into four different sections related to fish consumption habits and purchasing behaviour (1); perception of barriers to fish consumption (2); the importance of fish traceability, and knowledge of sustainability labels (3); collection of respondents social-demographic information (4). The estimated time for compilation was approximately 10 minutes.

A summary of the questionnaire questions is shown in Table 1. In the first section, it was asked to respondents their overall fish liking, by using a 9-point hedonic scale (1 = “Extremely disliking"; 9 = “Extremely liking”) (Claret et al., 2016) and their fish consumption frequency, by using a 7-point frequency scale (1 = “Never”, 2 = “Less than one per month”, 3 = “Once per month”, 4 = “2-3 times per month”, 5 = “Once a week”, 6 = “Twice a week” 7 = “More than two times per week”) (based on Hicks et al., 2008). Finally, respondents were asked about the status of the purchased fish (fresh, frozen, canned, and processed), by using a 7-points frequency scale with the following anchors: 1= “Never”, 4= “Occasionally”, 7= “Always” (Saidi et al., 2022).

In the second section, the perception of barriers to fish consumption was analysed. respondents were provided with a list of five barriers (high price, time required to prepare fish meal, no cooking ability, high perishability, no family preference as reported by Saidi et al., 2022;

Vanhonacker et al., 2010), to which they had to respond by using a 7-point relevance scale (1= "Not important"; 4= "Indifferent"; 7= "Absolutely important").

In the third section, the definition of food traceability as "The ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution" (European Parliament and Council, 2002) was provided to the respondents and by using a 7-points relevance scale (1= "Not important", 4= "Indifferent", 7= "Absolutely important") the importance of fish traceability was analysed (based on Rodriguez-Salvador & Calvo Dopico, 2023). Then by using a 5-point familiarity scale (Tuorila et al., 2001) anchored with 1= "I do not know it", 2= "I know it, but I never bought it", 3= "I bought it once" 4= "I bought it occasionally", 5= "I usually bought it ", respondents' knowledge about fish sustainability labels was also analysed. The sustainability labels involved in the questionnaire were the following: Aquaculture Stewardship Council (ASC), Best Aquaculture Practices (BAP), Friends of the Sea (FOS), and Marine Stewardship Council (MSC).

In the last section, respondents' socio-demographic data were collected. In particular, it was asked about gender, age, living area, education level, annual income, number of family members, number of children in the family, and diet habits.

Table 1. Summary of questionnaire

Section	Question	Scale	Answer	References
1	Fish liking	1-9 hedonic scale	(1) Extremely disliking - (9) Extremely liking	Claret et al., 2015
	Consumption frequency	1-7 frequency scale	(1) Never, (2) less than one per months, (3) once per months, (4) 2-3 times per months, (5) once a week, (6) twice a week, (7) more than two times per week	Hicks et al., 2008
	Purchased status (fresh, frozen, canned, and processed)	1-7 frequency scale	(1) Never, (4) Occasionally, (7) Always	Saidi et al., 2022

2	Barriers to consumption (high price, time required to prepare fish meals, no cooking ability, high perishability, no family preference)	1-7 relevance scale	(1) Not important, (4) Indifferent, (7) Absolutely important)	Saidi et al., 2022; Vanhonacker et al., 2010
3	Importance of fish traceability	1-7 relevance scale	(1) Not important, (4) Indifferent, (7) Absolutely important)	Rodriguez-Salvador and Dopico, 2023
	Knowledge of fish sustainability labels (ASC, BAP, FOS, MSC)	1-5 familiarity scale	(1)I do not know; (2) I know it, but I never bought it; (3) I bought it once; (4) I bought it occasionally; (5) I usually bought it	Tuorila et al., 2001
4	Collection of socio-demographic information		Gender, age, geographical living area, educational level, annual income, number of family members, number of children in the family, diet habits	

2.3. Data analysis

To properly decide the statistical approach to be used, normality test (Shapiro-Wilk test, $p>0.05$) was applied and the homogeneity of variance was tested as well (Bartlett's test, $p>0.05$). Paired samples t-test was used to find differences in the knowledge of sustainability labels between Italian and Spanish respondents. Analysis of the variance (one-way ANOVA and Duncan test, $p\leq0.05$) was used to find differences in the importance of traceability between the two countries, and to analyse the effect of social-demographic variables on consumers' responses. Then respondents were grouped by country of origin, gender, and age group, for a total of 16 observations and subjected to principal component analysis (PCA) to study the relationships between the observations and the variables analysed in the online questionnaire. Significance criteria were set at alpha=0.05. The XLSTAT statistical software package (version 2016.02, Addinsoft) was used for data analysis.

3. Results and discussion

3.1. Research overview

Table 2 describes the individual information for Italian and Spanish respondents. They were equally distributed for gender, with an age range from 18 to 70 years old (average of 45 y.o.) both in Italy and Spain. The respondents were classified into four age groups: 18-29; 30-44; 45-54; 55-70 according to Predieri et al., (2020) and Spinelli et al., (2021). Regarding geographical living areas, respondents from the seaside, and internal areas were involved. Regarding education level, the questionnaire included respondents with a middle school diploma, high school diploma, bachelor's degree, and master's or Ph.D. degree. Regarding annual income, respondents with an income from <20.000€ to >100.000€ were involved in the questionnaire. Finally, regarding diet habits, most of the respondents were omnivorous, and flexitarian, and only fish eaters completed the questionnaire.

Table 2. Characteristics of Italian and Spanish respondents involved in the questionnaire (%).

	Italy			**Spain**		
	Respondents n(%)	Female n(%)	Male n(%)	Respondents n(%)	Female n(%)	Male n(%)
Age						
18-29	167(17)	82(49)	85(51)	218(23)	83(38)	135(62)
30-44	274(29)	137(50)	137(50)	248(26)	104(42)	144(58)
45-54	230(24)	115(50)	115(50)	145(15)	91(63)	54(37)
55-70	290(30)	149(51)	141(49)	341(36)	200(59)	141(41)
Provenance						
Seaside	410(43)	203(49)	207(51)	521(55)	263(50)	258(50)
Internal	551(57)	280(51)	271(49)	431(45)	215(50)	216(50)
Education						
Middle school diploma	102(11)	52(51)	50(49)	105(11)	49(47)	56(53)
High school diploma	507(53)	247(49)	260(51)	375(39)	189(50)	186(50)
Bachelor	288(30)	148(51)	140(49)	368(39)	182(49)	186(51)
Master/PhD	64(6)	36(56)	28(44)	104(11)	58(56)	46(44)
Job						
Freelancer	107(11)	45(42)	62(58)	76(8)	44(58)	32(42)

Employee	385(40)	172(45)	213(55)	383(40)	203(46)	180(64)
Worker	110(12)	39(35)	71(65)	95(10)	47(50)	48(50)
Unemployed	187(19)	132(71)	55(29)	202(21)	97(48)	105(52)
Student	73(8)	41(56)	32(44)	90(10)	28(31)	62(69)
Other	99(10)	54(54)	45(46)	106(11)	59(56)	47(44)
Annual income						
<20.000€	257(26)	147(57)	110(43)	295(31)	116(39)	179(61)
20.000-40.000 €	395(40)	180(47)	215(53)	395(41)	215(54)	180(46)
40.000-60.000 €	123(17)	54(44)	69(56)	128(13)	75(59)	53(41)
60.000-100.000 €	38(4)	14(37)	24(63)	51(5)	38(74)	13(26)
>100.000 €	10(1)	7(70)	3(30)	15(3)	10(67)	5(33)
Not answer	138(12)	81(59)	57(41)	68(7)	24(35)	44(65)
Diet habits						
Omnivorous	832(87)	417(50)	415(50)	536(57)	302(56)	234(44)
Flexitarian	55(6)	33(60)	22(40)	110(11)	45(41)	65(59)
Only eat fish	62(6)	25(40)	37(60)	36(4)	20(56)	16(44)
Other	12(1)	8(67)	4(33)	270(28)	111(41)	159(59)

3.2. Respondents' interest in Traceability and Sustainability label

The interest in food traceability is becoming increasingly important for consumers during purchases. The fish sector, especially for the processed species (frozen, canned, refrigerated, etc.), has a very long and complex supply chain (Giusti et al., 2019), therefore, as highlighted by other authors, receiving information regarding fish traceability can improve individuals' trust, make consumers aware of this topic, and finally push them to buy tracked alternatives to untracked ones (Menozzi et al., 2015; Rodriguez-Salvador & Calvo Dopico, 2023). Furthermore, since fish is a highly perishable food (Prabhakar et al., 2020; Rodriguez-Salvador & Dopico, 2020), receiving detailed information about traceability can both help consumers when purchasing, reducing food scares, can also help food companies manage food-related issues (recalls of non-conforming products, problems related to specific production batches) (Menozzi et al., 2015). The results of this study showed that it is important for Italian respondents to receive information about the traceability of the purchased fish species. On the

contrary, for Spanish respondents, traceability information is less taken into account during the purchase. As shown in Figure 1, 41% of Italian respondents assigned a score of 7 on the 7-point Likert scale, while in Spain only 22% of respondents assigned the same score. Our results are consistent with another study reported that Spanish consumers have a lack of knowledge about fish traceability, so it is not a factor that is taken into account when purchasing fish products (Rodriguez-Salvador & Dopico, 2020). Moreover, it was found that to improve awareness of traceability, it is important to educate respondents about the practice but also to make traceability reporting more prominent on packages so that it is more easily visible and recognizable (Calvo Dopico et al., 2016). Applying ANOVA (one-way ANOVA and Duncan test, $p \leq 0.05$), statistically significant differences between the two countries were found ($p \leq 0.05$). Therefore, in response to research question one (RQ1) formulated above, it could be concluded that for Italians it is important to receive information on the traceability of purchased fish species, whereas for Spanish respondents this information is not of key importance at the time of purchase.

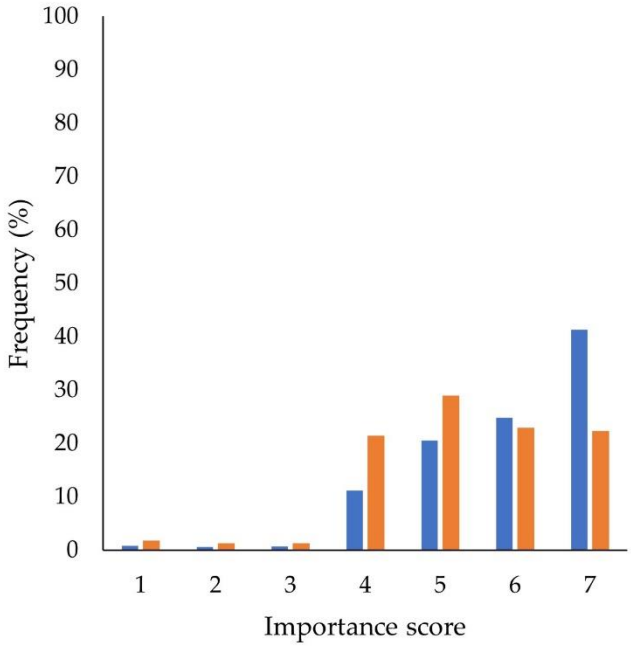

Figure 1. Importance of fish traceability in Italy and Spain. ■ Italy, ■ Spain

However, taking into account familiarity with sustainability labels, it was found that both Italian and Spanish respondents were unfamiliar with this issue. Indeed as reported in Figure 2 the answer with a higher percentage was always "I do not know it" (average response rate 58%), followed by "I know it but I never bought it" (average response rate 23%), and finally "I buy it regularly/occasionally" (average response rate 19%). Paired-samples t-test showed statistically significant differences between Italian and Spanish respondents among the different responses for the sustainability labels considered ($p \leq 0.05$) The best-known label is the Marine Stewardship Council (MSC), although the frequency of purchase is still low, with values of 31% and 28% in Italy and Spain respectively. So in response to research question two (RQ2), although interest in fish sustainability is increasing (Pucci et al., 2020), knowledge and consequently purchase of sustainable fish products is still low for both Italian and Spanish respondents. As confirmed in other studies (García-Herrero et al., 2019), the lack of information on this issue implies a lack of awareness about the sustainability of fish, which results in low purchasing power for this category of products.

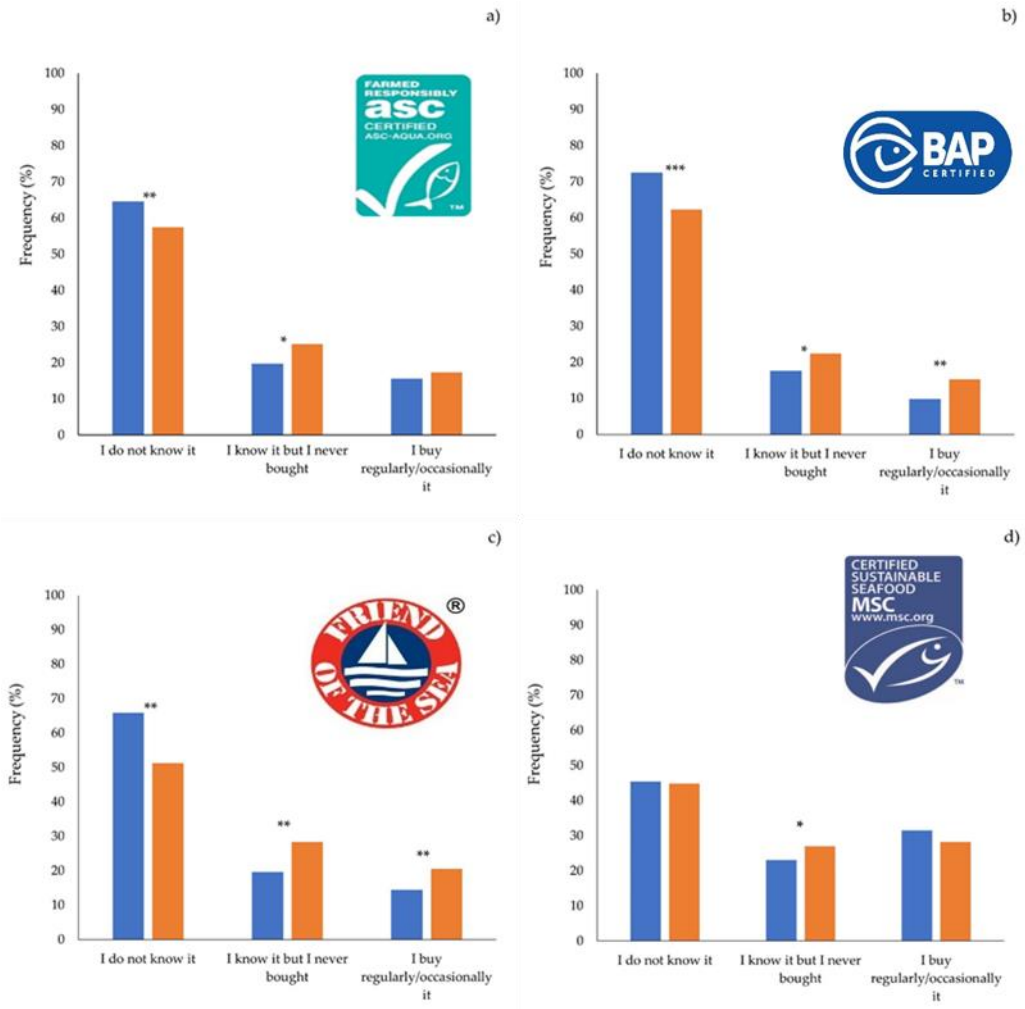

Figure 2. Sustainability label knowledge in Italy and Spain: ASC (a), BAP (b), FOS (c), MSC (d). ■ Italy (963), ■ Spain (954). Asterisks indicate significant differences (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

3.3. Effect of socio-demographic variables

The effect of socio-demographic variables on the questions is reported in tables 3a-b, for Italian and Spanish respondents, respectively (one-way ANOVA and Duncan test, $p \leq 0.05$).

Table 3a. Effect of social-demographic variables (average values ±SD) for Italian subjects.

Question	Gender			Age					Geographical location		
	F	M	p-value	18-29	30-44	45-54	55-70	p-value	Sea	Internal	p-value
Importance of traceability	6.0±1.1	5.8±1.2	0.072	5.6±1.2^{a}	5.8±1.3$^{a\,b}$	5.9±1.2^{b}	6.2±1.0^{c}	0.001	6.0±1.2^{b}	5.8±1.2^{a}	0.144
MSC	2.4±1.5	2.4±1.4	0.859	2.6±1.4^{b}	2.6±1.5^{b}	2.2±1.4^{a}	2.2±1.4^{a}	0.001	2.5±1.5	2.3±1.4	0.149
ASC	1.8±1.2	1.7±1.2	0.493	2.1±1.4^{c}	1.9±1.3^{b}	1.5±1.1^{a}	1.6±1.1^{a}	0.001	1.9±1.3^{a}	1.7±1.1^{b}	0.009
BAP	1.5±1.0	1.6±1.0	0.114	1.8±1.1^{c}	1.6±1.1^{b}	1.3±0.8^{a}	1.4±0.9^{a}	0.001	1.7±1.1^{a}	1.4±0.9^{b}	0.001
FOS	1.7±1.2	1.8±1.2	0.529	2.0±1.3^{b}	1.8±1.1$^{a\,b}$	1.6±1.1^{a}	1.7±1.2^{a}	0.020	1.8±1.2^{a}	1.7±1.2^{b}	0.044

For each column, different letters correspond to statistically significant differences (Duncan test $p \leq 0.05$).

Table 3b. Effect of social-demographic variables (average values ±SD) for Spanish subjects.

Question	Gender			Age					Geographical location		
	F	M	p-value	18-29	30-44	45-54	55-70	p-value	Coastal	Inland	p-value
Importance of traceability	5.3±1.3	5.4±1.2	0.074	5.1±1.3^{a}	5.4±1.2^{b}	5.6±1.1^{b}	5.5±1.3^{b}	0.001	5.3±1.3	5.3±1.3	0.950
MSC	2.3±1.4^{a}	2.5±1.4^{b}	0.034	2.7±1.4^{b}	2.7±1.4^{b}	2.1±1.5^{a}	2.1±1.4^{a}	0.001	2.4±1.4	2.3±1.4	0.881
ASC	2.0±1.2	2.0±1.3	0.155	2.3±1.3^{b}	2.2±1.3^{b}	1.8±1.3^{a}	1.6±1.1^{a}	0.001	1.9±1.2	1.9±1.3	0.624
BAP	1.8±1.2	1.9±1.3	0.078	2.2±1.3^{b}	2.1±1.3^{b}	1.7±1.2^{a}	1.5±1.0^{a}	0.001	1.7±1.1	1.9±1.3	0.113
FOS	2.0±1.3	2.2±1.3	0.065	2.5±1.3^{b}	2.4±1.4^{b}	1.9±1.3^{a}	1.7±1.2^{a}	0.001	2.2±1.3	2.0±1.3	0.644

For each column, different letters correspond to statistically significant differences (Duncan test $p \leq 0.05$).

As shown in Table 3a, gender did not influence the importance of traceability and awareness of sustainability labels in Italian respondents; ANOVA showed no statistically significant differences ($p \geq 0.05$). Whereas in Spanish respondents, men were found to be more aware only of the MSC sustainability label ($p \leq 0.05$). The age of the respondents inversely influenced the importance of traceability and knowledge of sustainability labels in both Italy and Spain. ANOVA showed that as age increases, traceability information becomes more important for respondents ($p \leq 0.05$). The results are in agreement with Myae & Goddard, (2012) who point out that traceability is more important for adults, who are more careful about the safety, freshness and quality of the products they used to buy. Conversely, as age decreases, knowledge of sustainability labels increases with statistically significant differences ($p \leq 0.05$). This last result is in line with the evidence in the literature which reported significantly higher perceived importance of sustainability among young consumers (ranging from 20-40 years old) (Salladarré et al., 2016; Verbeke et al., 2007). Many studies report a positive consumer willingness to buy sustainable fish once informed about sustainability labels and their meaning (Bronnmann & Asche, 2017; Groening et al., 2018). This consideration suggests that young respondents are more informed about the meaning of sustainability labels, and being more aware of their importance, they are also more used to buying sustainable fish options.

Applying ANOVA to find differences due to the geographic origin of the respondents (coastal or inland area), it was found that in Italy this variable only influences knowledge of the ASC, BAP, and FOS labels with statistically significant differences ($p \leq 0.05$). On the other hand, the importance of traceability and knowledge of the MSC label is greater for respondents from seaside areas but without showing a statistically significant difference ($p \geq 0.05$). In Spain, on the contrary, geographical origin does not show statistically significant differences in traceability and fish sustainability ($p \geq 0.05$). In response to research question three (RQ3), respondents' personal information influences their interest in fish traceability and sustainability.

In particular, the results show that age has a greater influence on respondents' perceptions of the variables discussed.

3.4. Principal component analysis (PCA)

Table 4 reported the correlation between variables and factors. Figure 3 is a representation of the respondents on the first two dimensions extracted by PCA accounting for 66.56% of the variance (38.42% PC1; 28.14 PC2). Respondents were well separated in terms of provenance and age, indeed Italian respondents are located in the upper part, while the Spanish respondents are located in the lower part of the graph; as well as, the younger respondents are located in the right part, while the adults are in the left part of the graph. Figure 3 allows observing the variables which characterize the respondents and the associations among them. The results showed that adult Spanish respondents consume more frequently fresh fish than Italian ones, indeed, as reported in another study (Rodriguez-Salvador & Calvo Dopico, 2023), Spain is among the top nations in fish consumption in Europe. On the other hand, adult Italian respondents have shown a greater liking for fish products compared to Spanish, they also buy more frozen fish and perceive the high price and the high perishability as a fish consumption barrier. It is well established that fish consumption is beneficial to health, but the recommended daily intake is not widely achieved, this is due to fish consumption barriers (Altintzoglou et al., 2011; Burger & Gochfeld, 2009; Grieger et al., 2012). As reported by other authors (Leek et al., 2000; Olsen, 2001; Verbeke & Vackier, 2005) fish price is the main barrier to consumption frequency. Although the price increase is often interpreted as better quality, Claret et al. (2014) report that the increase in the price of fish products reduces the willingness of consumers to buy these products or to buy them in smaller quantities. Regarding the high perishability, fish are highly perishable food products due to their composition, which involves a series of chemical and microbiological reactions that take place from the post-catch phase until final consumption; this phenomenon is called autolysis (Hicks et al., 2008; Prabhakar et al., 2020; Rodriguez-Salvador & Dopico, 2020). Thus, the tendency to buy frozen products may be due to the

perceived barriers among Italian adults. Such products, unlike fresh products, receive minimal processing treatment (cutting, filleting, removal of non-edible parts and finally wrapping), so they also come with more information than fresh fish products. This could justify the Italian respondents' greater interest in fish traceability compared to Spanish respondents, who by buying more fresh fish, are more used to receiving less information about what they buy and are therefore better able to self-identify the characteristics of the purchased species. However, some fraud may be related to the provenance and freshness of the species purchased (Cutarelli et al., 2014; Giusti et al., 2019), so it would also be advisable to disseminate strategies to raise awareness of fish traceability to direct purchases towards this category of products and reduce the probability of any kind of fraud.

Young respondents, especially Italians, on the other hand, perceive other types of barriers to consumption, such as the inability and time required to prepare a fish meal and the low preference for fish in the family. This is why they tend to buy more transformed products that are tasty and easy to prepare. Such products are currently the only ones on the market with sustainability labels, which is why this class of consumers may be more aware of such labels. As reported above, although this respondents' class is more informed about sustainability labels, awareness of such labels is still very low. As discussed above, awareness-raising strategies are needed to steer consumer purchases toward more sustainable choices.

Table 4. Correlation between variables and factors

Variables	**F1**	**F2**
Fish liking	-0,712	0,380
Consumption frequency	-0,225	-0,864
Purchase status Fresh	-0,522	-0,643
Purchase status Frozen	-0,356	0,222
Purchase status Canned	-0,735	-0,087
Purchase status Transformed	0,234	0,923
Barriers Price	-0,532	0,061
Barriers Time	0,367	0,888

Barriers No cooking ability	0,575	0,652
Barriers High perishability	-0,243	0,501
Barriers No family preference	0,677	0,208
Importance of traceability	-0,649	0,625
MSC	0,876	-0,137
ASC	0,892	-0,379
BAP	0,829	-0,460
FOS	0,812	0,119

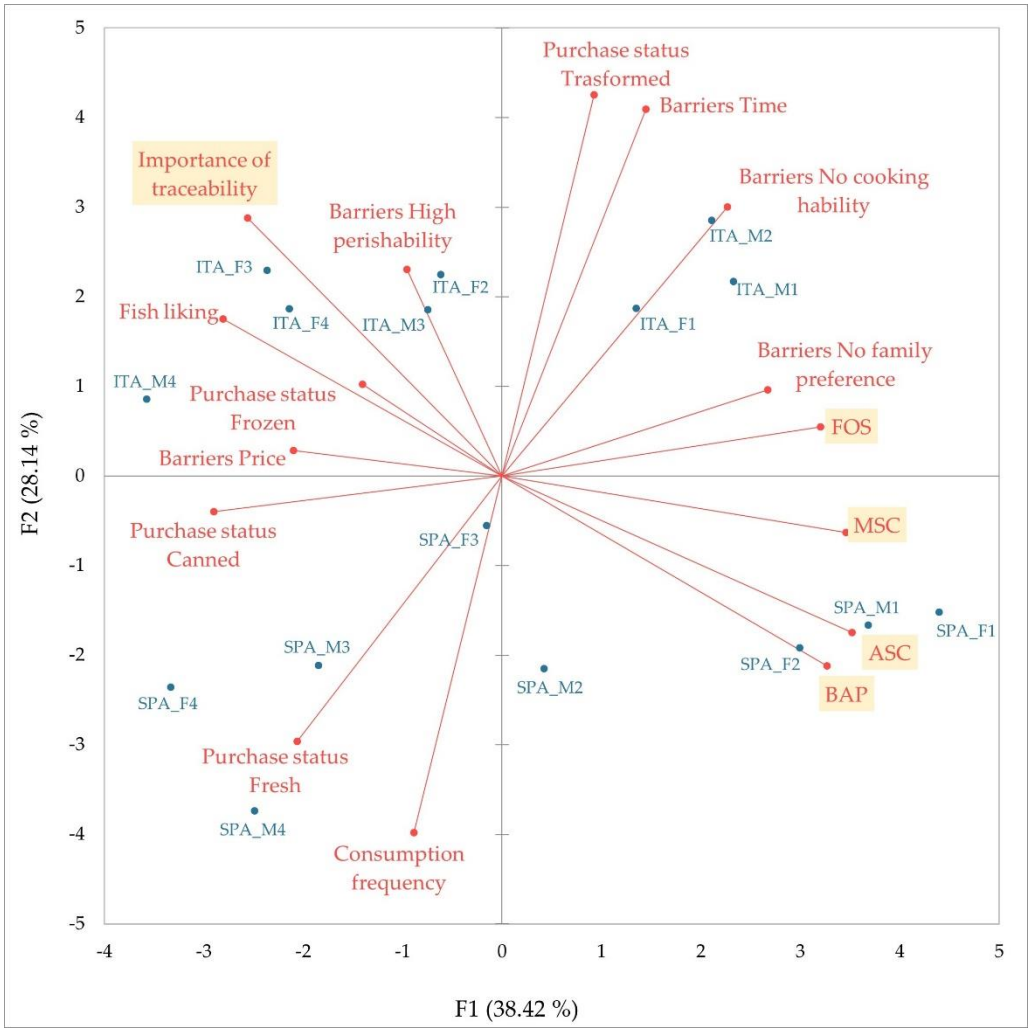

Figure 3. Principal component analysis (PCA) plot of 16 groups (provenance, gender, age) of respondents (observations) and 16 variables (x variables). 🔴 Active variables, 🔵 Active observations. ITA (Italian); SPA (Spanish); M (Male); F (Female); Numbers from 1 to 4 are related to age group (1: 18-29, 2: 30-44, 3: 45-54, 4: 55-70).

4. Conclusions

The study was based on three research questions related to the importance of traceability (RQ1), knowledge of sustainability labels (RQ2) and the influence of socio-demographic variables (RQ3) in the respondents' choices and knowledge. The questionnaire highlighted that traceability is more important to Italian than to Spanish respondents. These differences are due to consumption and purchasing habits as well as the personal variables of the respondents. Sustainability labels, on the other hand, were little known in both countries. Younger respondents, given their purchasing habits, were found to be more informed about fish sustainability issues. Therefore, dissemination strategies are necessary to improve knowledge about traceability and fish sustainability to orientate purchases towards this category of products and to value companies that provide accurate and detailed information and that respect both animal and environmental sustainability. Considering the results, market strategies oriented toward traceable and sustainable food choices can be based on the individual characteristics found to influence the consumers' knowledge about traceability and fish sustainability. A possible limitation of the study is that the respondents' opinions on fish traceability and sustainability were only analysed through an online questionnaire. Future research may focus on consumer tests to analyse the impact of such variables on the perception of different fish products during sensory evaluation by consumers.

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Chapter 7

Conclusion and future prospective

With the progress of time and the increased consumer demand s on what they buy, the application of sensory science is useful for the evaluation of food, as well as for products and services in general.

This PhD thesis aimed to apply sensory procedures to increase knowledge in the field of seafood, especially for Mediterranean species, for both companies in the sector and consumers. In addition, sensory methods were developed to ensure the quality, authenticity and provenance of the studied species and to learn about consumers' perceptions and needs regarding fish products.

In particular, as explained in Chapter 2, sensory evaluation was used to verify the authenticity of fish species. Through the use of the descriptive analysis, a panel was trained to find the typical attributes, characterise and discriminate between different and differently obtained (wild-caught and farmed) fish species and crustaceans, thus showing the potential of sensory analysis as a tool to find the peculiar characteristics of the species and thus avoid possible commercial frauds. Future studies could characterise other species to create classification charts of the most common fish species, available online or in fishmongers to help consumers when buying fresh fish, especially when the fish is sold in slices or fillets and therefore the peculiar characteristics of the species are difficult to distinguish. A possible limitation of using descriptive analysis is the need for extensive panel training, which requires time and resources from the judges involved.

Subsequently, in Chapter 3, it was shown how sensory evaluation can be used to assess fish freshness. In particular, through the use of the quality index method, QIM, the assessment of freshness can be quickly and objectively done, although the method is species-specific and training on the method and rating scales are necessary. In addition, the effect of an electrostatic

field to improve the quality along the shelf-life of highly perishable species was also evaluated, showing the potential of this technology, although it needs to be further optimised. Indeed, further studies could concern the application of the DENBA+ electrostatic field during frozen storage temperatures, as well as applying this technology to other fish species, of different sizes to check whether the effect of the electrostatic field could be influenced by species characteristics. Moreover, other instrumental parameters, such as colour and pH could be measured to better understand the effect of this preservative non-thermal technology.

Other applications of sensory science, which do not directly involve tasting or evaluating a product, are online questionnaires. Indeed, as described in Chapter 4, an online questionnaire was used to assess the knowledge and propensity toward seafood traceability and sustainability of two coastal countries with high seafood consumption. This chapter shows great interest in the issues of sustainability and fish traceability, although they are not fully known by consumers in both countries. Moreover, subjects' sociodemographic variables influenced their purchasing habits and thus their propensity toward sustainability and traceability issues. Further studies may concern consumer tests in the presence to verify the hedonic acceptance of species with and without sustainability labels and with traceability information.

By exploiting the results of Chapter 4, a consumer test was carried out with Italian consumers to test the impact of a sustainability label on seafood liking (Chapter 5). The results of this activity showed that consumers, probably due to a lack of knowledge about fish sustainability, increased their liking scores when aware that the species tasted was accompanied by the sustainability label, compared to the species without the label. The highest scores were recorded during the expected session, thus demonstrating a higher expectation of liking sustainable products than when the products were evaluated in the informed session. Thus this chapter highlights the external conditioning of the extrinsic variables provided at the time of purchase.

Following this line, Chapter 6 reports the use of a questionnaire to test the impact of external information for Red King Crabs in different packaging settings on Northern European consumers. Check all that apply was used to collect information concerning labelling and origin of red king crab. The results of this chapter showed that consumers interviewed associated positive attributes with crab settings with information regarding traceability and sustainability, and negative attributes compared to those with less information. Thus, highlighting a higher propensity to buy and willingness to pay for species with more information.

In conclusion, sensory science is useful along the fishing supply chain in several aspects. Indeed, sensory procedures have proved useful to characterise and verify the authenticity of fish species, to assess freshness, and to know consumers' attitudes towards fish-related information such as traceability and sustainability. From future perspectives, the consumer knowledge in the fish field should be improved through dissemination strategies, creation of websites or smartphone apps useful for consumers to quickly check the quality, and authenticity of species, as well as consult information regarding sustainability labels and their meaning. In this way, then, subjects can improve their knowledge and make more informed purchases, but most importantly, prevent the high consumption of fish and seafood products from impacting the environment too negatively.