ADVANCES IN AQUACULTURE AND FISHERIES RESEARCH

Editors
Prof. Dr. Aysun KOP
Assist. Prof. Dr. Boran KARATAŞ

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PREFACE

Aquaculture, which is one of the most important components of the agricultural sector and has a high production potential, is one of the strategically important sectors in the world economy. In this context, there has been non-negligible increases in studies in the field of aquaculture in recent years. Sustainability of fisheries resources, which is essential in today's world, is possible with the use of data obtained as a result of scientific studies. Herein each study contributes to the development of the aquaculture sector.

This book is designed to evaluate the current situation, current developments and new approaches in the field of aquaculture. The purpose of this book is to contribute to the development of the sector by addressing the theoretical and practical issues in different fields of aquaculture. The presented book has been prepared with the contributions of various researchers in the field of aquaculture from several universities and consists of thirteen chapters.

We sincerely hope that the current book, which includes reviews and researches, will be useful to the fisheries industry, its students, researchers conducting scientific studies in related fields, and science world. We would like to thank the esteemed chapter authors who contributed to the preparation of the book and contributed with their professional experience in the individual chapters, technical team, and İKSAD Publishing House, which brought such a book to the scientific world.

EDITORS

CHAPTER 1

THE APPLICATION OF ELECTROSPINNING TECHNOLOGY AND ELECTROSPUN NANOFIBRES FOR FOOD AND FISHERY PRODUCT PACKAGING

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1. INTRODUCTION

Microbial contamination is the most significant issue affecting the quality and safety of food products in the food industry (Liu et al., 2022). The shelf-life of food products is influenced by a variety of factors, primarily microbial phenomena, resulting in a significant decrease in consumer acceptance. Therefore, packaging technologies are very critical for preventing spoilage and extending the shelf-life of food and fishery products. There has been a growing interest in using plant-based bioactive ingredients instead of synthetic preservatives in recent years. However, when this substitution is fully implemented, their low resolutions, short-term activities, low chemical stability, and off-sensorial characteristics, as well as some difficulties with bioactive components, are discovered. So, encapsulation can overcome these constraints (Hosseini and Jafari, 2020). Electrohydrodynamic techniques are used to improve structures intended for encapsulation. Their unique properties provide numerous significant benefits for nutraceutical and food applications. Improved zein microstructures are critical materials for encapsulating bioactive components for medical, nutraceutical, and functional food applications (Coelho et al., 2022). Carbonhydrate or protein-based encapsulating materials have been used not only in electrospinning, but also in the electrospinning encapsulation of probiotics (Ghorani and Tucker, 2015). Many benefits of using electrospun nanofibers and encapsulating food bioactives for food and fish packaging have been discovered. These are room temperature procedures that use food-grade biopolymers and polymers to achieve controlled release, effective encapsulation, reduced denaturation, and improved bioactive stability. Furthermore, the size of the final product can be checked by controlling processing parameters (Ghorani and Tucker, 2015).

Electrospinning is a novel technique that is regarded as a sophisticated and highly efficient method for producing nanofibres with numerous functional properties and usable structures (Min et al., 2022b). Nanofibers are defined as having a mean diameter less than 100 nm. The "electrospinning technique" is the simplest and most efficient method of producing nanofibers (Terzi, 2013). This technique can be used not only for producing fibers of various sizes (Karabulut et al., 2019), but also for controlling the surface properties of nano-fibers to produce substrates and sensors for the rapid detection of various contaminants (Hajikhani and Lin, 2022). Nanotechnology has improved nano-

products with very different properties in recent years. Nanosensors are one of the most important nanotechnology products developed. Nanosensors, which were previously used in fields such as health, medicine, and electronics, are now finding a significant and novel application in the food industry. Nanosensors not only provide a quick, cost-effective, and dependable measurement option, particularly for the element desired to be detected in food products, but they can also be designed using the electrospinning method, which produces nanometer-thin materials via electric field attraction. This method can thus be used to create functional nanoscale sensors with novel features (Ylmaz and Altan, 2017). Recently, electrospinning technology and electrospun micro/nano structures have many advantages or beneficial attributes such as cost-effectiveness, delivery cargos for various food bioactive compounds, bioactive release performance, tunable morphologies, the controllable degradation mechanism of the polymers, and matrix mechanical features (Rostamabadi et al., 2020).

Many studies have been conducted on recent advances in packaging technologies utilizing the electrospinning technique. To improve nanofibrous fish skin gelatin (FSG) from an aqueous precursor, a highthroughput alternating field electrospinning (AFES) process was used. According to preliminary findings, this is a green method for producing a biomaterial that is biocompatible, bioactive, and suitable for (1:1) scale production (Kennell et al. 2022). Researchers have investigated electrospun nonwovens loaded with volatile and nonvolatile substances. Food packaging is used in the food industry, and composite structures can interact with the packaging's changing external conditions during distribution or storage. Temperature abuse, microbial growth, increased relative humidity, and other factors all have an impact on food quality and shelf-life (Loong-Tak, 2021). Recent advancements in electrospinning as an encapsulating method for plant extract have improved food shelf-life (Munoz-Shugili et al., 2021). Packaging technologies are constantly evolving. As a result, recent advancements in food and fish products related to the applications of electrospinning and electrospraying have been discussed in this review, with a focus on summarizing the methods' characteristics. Furthermore, reviewing innovative processing techniques used in foods and seafood, as well as some of the benefits and drawbacks of these methods from the standpoint of food industries, has been highlighted.

2. ELECTROSPINNING TECHNIQUE AND PROCESS

The majority of the textile product qualities come from the fiber properties. Due to their superior handling, more appealing look, and high-performance characteristics, fibers with a higher degree of fineness are more desirable than coarse fibers when utilized in production. Traditionally, melt spinning, dry spinning, and wet spinning methods could be used to create synthetic filament. However, there is a certain range in which the molten polymer can be spun and stretched during the melt-spinning process. The incredibly tiny fibers known as "nanofibers" are currently quite popular, and nanotechnology has emerged as one of the most significant fields for both the academic and commercial worlds, extending these bounds. the process of "electrospinning" used to create nanofibers. One of the fundamental processes for creating fibers with nanometric dimensions is electrospinning. Using a receptacle, a pump, a high-voltage power source, and a collector, electrospinning is a method for creating nanofibers and particles by using a voltage process to a polymer solution.

By utilizing strong electrostatic forces, a polymer solution or melt can be electrospun into fibers with a reduced diameter. The applied potential voltage differential between a charged polymer and a grounded or oppositely charged collector causes the process to start under the influence of these electrostatic forces. Surface tension is overcome by the electrical force that forms at the surface of a polymer solution or polymer melt as a result of the applied voltage. The polymer drop then releases a charged jet. The jet initially travels in a straight line for a short distance before bending and swirling. The liquid or melt solidifies when the electrical forces lengthen the jet hundreds or even millions of times, making it incredibly thin. Finally, extremely long nanofiber gathers on a collector that is grounded electrically. This method can be used to produce extremely tiny fibers with diameters less than 10 nanometers (nm) (Daşdemir, 2006).

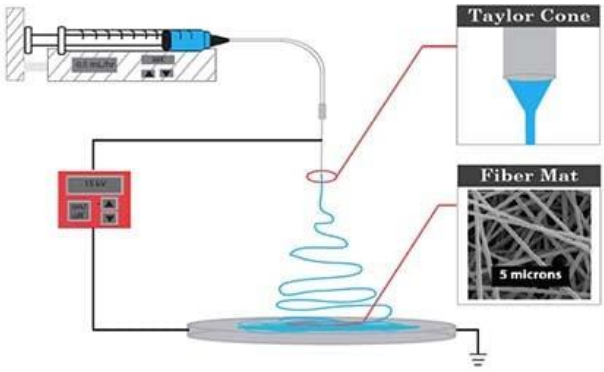

Figure 1. Electrospinning principle diagram (https://www.nanofiberlabs.com/faq.html)

Electrospinning process consist of a high-voltage power source, a container for a polymer solution or melt with a small opening to be utilized as a nozzle, and a conductive collection device. Either by directly immersing the electrode in the polymer solution or melt, an emitting electrode of the high-voltage power source charges the polymer solution or melt (Huang et al, 2003).

Electrostatics, polymer science, and fluid mechanics are all intricately intertwined in the electrospinning process. The process can be significantly impacted by a number of variables such as; the characteristics of spin dope, the process variables and the environmental conditions. The whole understanding of electrospinning is still being studied at this time, and complete processing control is still lacking (Yichun, 2019).

Electrospun nanofibers have prospective uses in environmental engineering and biotechnology, such as gas/liquid and molecular filtration, thanks to the rapid growth of nanoscale science and technology. Industrial uses for energy and electronics like nanoelectronics, electromagnetic interference shielding, nano solar cells, LCDs, extremely light-weight materials for spacecraft, and more effective catalysts, capacitors, and battery separators. Defense and security measures include piezoelectric, thermal, and other nanosensors as well as military protective gear. For tissue engineering scaffolding, applications in the biological sciences, and cosmetic skin masks, bioengineering and medical science (Angammana, 2016). More than half of applications are for the medical prosthesis, which is the most popular use (Huang, 2003).

3. PRODUCTION METHODS OF NANOFIBERS

Since it is not a new method and has been used for more than three hundred years, electrospinning has been the focus of much research (Kataphinan 2004). Rayleigh first saw the electrospray method in 1897, and Zeleny thoroughly investigated it in 1914. The electrospinning produce the fibers by using an electric field and electrostatic force (Thandavamoorthy et al, 2005).

Despite the fact that there have been several studies on electrospinning, it has been noted that there have only been a small number of investigations into the electrospinning of nanofibers. There are numerous classes based on the original fluids, equipment types, and production scales. Electrospinning production methods could be made from polymer solution or melt. The main research focus is currently on solution electrospinning at room temperature because to its simple technology, as opposed to melt electrospinning, which needs an additional heating unit and may operate at high temperatures. However, melt electrospinning is a great alternative for polymers without suitable solvents because it offers the prospect of commercial application and has no pollution problems associated with solvent evaporation (Guangdi, 2019). The use of polymer melt in electrospinning has distinct advantages compare to solution electrospinning. These advantages could be listed as follows:

- In the melt electrospinning process, there is no environmental contamination, due to no need for solvent.
- Additionally, melt electrospinning can be used to handle some polymers, including PE and PP, that are not well solvated at room temperature.
- Third, some solutions of thermoplastic polymers can be electrospun, such as; polycaprolactone (PCL) and polylactic acid (PLA). These are also suitable for melt electrospinning, which will significantly boost fiber yields with high levels of precursor usage and do away with residual solvent in finished goods (Guangdi, 2019).

Numerous variables can affect the morphology of the formed fibers. The important production parameters are; the syringe pump flow rate, the concentration of the polymer solution, the type of collector, the viscosity of

solution, the applied voltage, the distance between the collector and the nozzle, the diameter of the nozzle, and etc.. The each of these elements has a big impact on fiber morphology. The decrease of the polymer concentration could cause electrospraying. In addition to this, the increase of the voltage can lower the fiber diameter. Therefore, in order to produce fibers with the highest performance, all of these parameters had to be optimized before the spinning process started (Bayrak, 2022).

Single-needle Electrospinning

Single-needle electrospinning is the original and arguably most popular electrospinning method. This method, based on single-needle equipment, appears to be straightforward but is actually quite complex and is subject to extensive influences from processing parameters, spin dope characteristics, and environmental factors (Figure 2.) Based on the jet formation and the way of using the needles, electrospinning methods can be classified as; single-needle, multi-needle (multi-nozzle), and needleless electrospinning (Zhou et al. 2009).

Multi-needle Electrospinning

Several needles or nozzles are employed as spinnerets in the multi-needle electrospinning technique, each holding one or more than one type of polymer solutions. After a high voltage is given to the needle's tip, nanofibers are accumulated on the collector The main benefits of multi-needle electrospinning include increased productivity and the ability to combine several polymers in the exact proportions needed to create hybrid nanofiber mats and membranes.

Figure 2. Linear multi-needle and multi-coaxial electrospinning spinneret
(https://www.electro-spinning.com/tubeless_spinneret.html)

The simplest multi-nozzle electrospinning setup features a spinneret with two nozzles, and when the two nozzles are positioned coaxially, the setup can be utilized to create core-shell nanofibers. Other sorts of structures that can be produced by multi-nozzle electrospinning include hollow and multi-channel nanofibers.

Needleless Electrospinning

The most significant problem in the creation of nanofibers is the poor production speed of the electrospinning technology, which severely restricts commercial uses. Although multi-needle electrospinning can significantly

increase productivity, industrial-scale production is still a long way off. An important alternative method for scaling up the manufacturing of nanofibers is needleless electrospinning.

Needless electrospinning has some advantages to needle electrospinning, including the ability to significantly increase nanofiber productivity and the avoidance of the needle clogging issue. On the other hand, it can be challenging to accurately regulate the morphological characteristics of the generated nanofibers. This is due to the fact that the electric field distribution in needleless electrospinning is more complex than it is in needle electrospinning, and that the spinneret's shape and geometry as well as the characteristics of the spin dope also have a big impact (Yichun et. al., 2019).

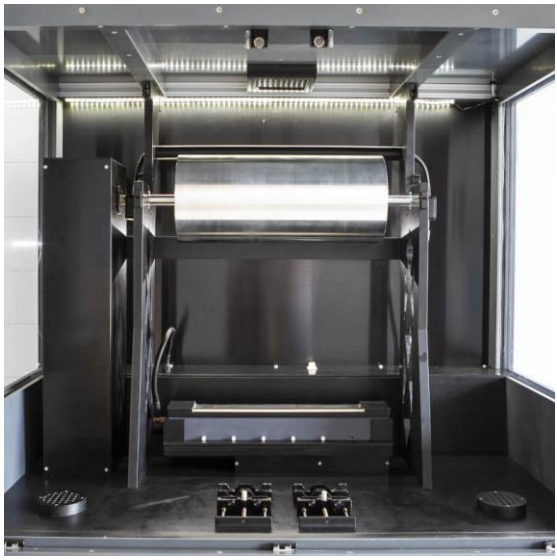

Figure 3. Electrospinning Needleless System
(https://www.ske.it/products/ef500-needleless-system/)

The Classification of Needleless Electrospinning

The needleless electrospinning techniques can be classified into two primary groups, such as free surface spinning and spinneret spinning, depending on the mechanism used to generate fibers, the spinneret's motion, and the direction in which fibers are collected.

The two primary categories of free surface spinning are free solution spinning techniques and free bubble spinning techniques. When using the

bubble-spinning technique, the porous surface positioned at the bottom of the polymer solution serves as the air source. The bubble forms at the polymer solution's surface, and the jet develops at the bubble's charged surface. Additionally, two layers were used in the free-surface spinning setup: high voltage was used, electrified jets underwent intense electric field stretching, the solvent evaporated, and formed nanofibers were then deposition on the upper counter electrode (Munir, 2020).

There are two primary categories of spinneret spinning: stationary spinnerets and revolving spinnerets. The high voltage is provided to a stationary spinneret, and a unique mechanism is used to feed polymer solution onto the spinneret. On the margins of a stationary spinneret that creates nanofibers, polymer jets form. According to the spinneret position, stationary spinnerets are further divided into three groups suc as; horizontal, downward, and upward stationary spinnerets (Munir, 2020).

In the rotating spinneret method, a polymer solution is licked into the surface of the spinneret as it is being turned. The spinneret forms polymer jets on its surface and nanofibers that are deposited on the collector in case of high voltage is applied to it. Three varieties of stationary spinnerets that rotate are those that are horizontal, downward, and upward (Munir, 2020).

4. THE APPLICATION OF ELECTROSPINNING IN FOOD

Nanotechnology is thought to be used many areas of food science due to the growing desire for protection against foodborne illness and longer shelf-life of food products. This situation causes to the urging need to develop antimicrobial food packages (Kowsalya et al., 2019). Nanosensors have a very important place in terms of the usage of nanotechnology in food packaging. The composition and nutrition values of foods, freshness or toxic substances from contamination can be determined with nanosensors. Also, the food packaging supply chain with embedded nanosensors can determine the state of food in internal and external conditions throughout (Qureshi et al., 2012). Nanosensors have several advantages, including their low cost and speed. They also provide accurate measurements for use in food. They can also be created using the electrospinning method, which uses the gravity of an electrical field to create nanometer-diameter materials. Furthermore, electrospinning technology can be used to create functional nanosensors with novel properties for the food

packaging industry (Yılmaz & Altan, 2017). The quality and safety of food can be easily monitored thanks to the development of nanosensors (Duncan, 2011). According to one study, a glucose biosensor was created by electrospinning nylon nanofibers made from nylon-6 (NyNF). This sensor's detection unit and the enzyme glucose oxidase were covalently bound on the electrode's base by a glassy carbon coated with NyNF. This study's biosensor's glucose performance was compared to that of a colorimetric glucose kit. For glucose detection, processed milk, cola, and energy drinks were used. These glucose descriptors produced significant results in diabetes control (Scampicchio et al., 2010). An electronic tongue was created using electrospun nanofibers to detect tetracycline antibiotics in dairy products (Scagion et al., 2016).

For food packaging, the properties of the polymers, i.e. their special characteristics such as mechanical attributes, biocompatibility, and degradation behavior for electrospun fibers, are critical to consider (Rostamabadi et al., 2020). Electrospinning technology could be used to create nanomicrofiber structures of biopolymers with a wide range of mechanical properties, compositions, bioactivities, and morphologies (Mendes et al., 2017). Furthermore, electrospinning technology has been used to deposit electrospun fibers on films to improve their performance, such as reinforcing materials or barrier properties. They can even be protected the food from the antifungal, antimicrobial and antioxidant spoilage (Topuz and Uyar, 2020). Electrospun and electrosprayed materials have become increasingly important in food applications in recent years to extend the shelf-life and also to improve the quality and safety of foods. Nonwoven materials such as electrospun or electrosprayed materials that encapsulate various bioactive substances for various purposes have been infused with synthetic polymers and food-grade biopolymers (preventing undesirable interactions with other food ingredients, improving bioaccessibility, controlling the release profile of bioactive substances, increasing storage stability and shelf life, etc). Long-Tak (2021).

Numerous studies on the application of electrospun nanofibers to foods have been conducted. Using a needle-free electrospinning technique, the authors created rapidly soluble antioxidant nanofibers from gelatin and *Spirulina* protein concentrate (SPC). The effects of the gelatin (20% w/w) and SPC (10% w/w) mixing ratios were tested not only in terms of the morphology and diameter of the nanofibers, but also in terms of the electrical conductivity,

viscosity, and surface tension of the electrospinning solutions, with the result that the electrospun gelatin/ SPC nanofibers have good potential for packaging and food applications (Mosayabi et al., 2022). Chitosan, gelatin, and 3-phenylacetic acid (PLA) are used to create electrospun nanofibres with antibacterial properties that can be used to extend the shelf-life of chicken meat in the refrigerator. These antibacterial electrospun nanofibres inhibited foodborne pathogens such as *Staphylococcus aureus* (SA) and *Escherichia coli* (EC) in this study and extended the shelf-life of chicken meat to 4 days in refrigerated storage (Liu et al., 2022). The needleless electrospinning process improved zein nanofibers with tetradecane and cinnamaldehyde as antioxidants and antibacterial agents. According to the findings of this study, the nanofibers used in sausage packaging not only increased the shelf-life of the sausages but also inhibited the growth of *S. aureus* and *E.coli*. Furthermore, needleless electrospinning can preserve sausage texture, color, thiobarbituric acid, and peroxide levels (Karim et al., 2022). Another study used mint essential oil to prepare zein-based nanocomposite material. This prepared material with mint essential oil was found to be not only strengthened by chitin fibers/chitin microcrystals using electrospinning, but also more effective on pathogenic bacteria *S. aureus* than *E. coli* in this study. This study also claims that the nanocomposites created can be used to improve potential active packaging systems (Karabulut et al., 2019). Shaibani et al. (2016) also investigated the determination of *E. coli* using the pH-sensitive hydrogel nanofiber light addressable potentiometric sensor (NF-LAPS) (2016). Moisture-activated hexanal release from an imidazolidine precursor encapsulated in ethylcellulose-poly(ethylene oxide) nonwoven increased the shelf-**l**ife of papaya fruit in the food distribution chain by four days (Ahenkorah et al., 2020). Electrospun films enriched with ethyl lauroyl alginate extended the shelf-life of strawberries to 25^{o}C. (Li et al., 2021). Electrospun β-carotene-loaded soy protein isolate (SPI): polyvinyl alcohol (PVA) fiber mats were used as bioactive coatings for food packaging, which helped to modulate the active ingredient release. Stable 50:50 SPI:PVA emulsions were improved with soybean oil (SBO) as a carrier of hydrophobic β-carotene (Bruni et al., 2020). The antibacterial activity of stable silver nanoparticles (AgNPs) and silver-polyvinyl alcohol (PVA) nanofiber against the tested food pathogenic bacterial strains *Pseudomonas aeruginosa*, *Bacillus cereus*, *E. coli* and *S. aureus* was very good. When applied to *Fragaria*

ananassa (strawberry) and *Citrus limon* (lemon), AgPVA nanofiber extended shelf-life by preventing deterioration caused by foodborne pathogens (Kowsalya et al., 2019). The packaging material created by electrospinning polyvinyl alcohol (PVOH) with essential oils (EOs) from two commonly used spices (*Rosmarinus officinalis* (REO) and *Laurus nobilis* (LEO) was applied to chicken breast fillets to extend their shelf-life. When applied to chicken breast fillets, this packaging material inhibited microbiological growth and lipid oxidation and it had a positive effect on both color and pH parameters during storage (Goeksen et al., 2021). To improve the physical properties of the edible gluten film, optimal nanofibers were developed. When compared to a control sample, the edible film treated with nanofibers had lower water vapor permeability and better mechanical properties. The authors of this study stated that jetless electrospinning is a new technology that can be used to produce nanofibers on a large scale, which can then be used to improve the mechanical properties of edible films (Ebrahimi et al., 2019). The nanocapsulation of cinnamaldehyde with zein nanofibers was found to have a bactericidal effect on *E. coli* O157:H7 and *S. aureus* PTCC 1337, but no negative effect on the textural, sensory, and color properties of sausages was observed. The authors demonstrated in their study that encapsulated fibers can be used instead of nitrite in meat (Karim et al., 2021).

5. ELECTROSPINNING APPLICATIONS IN FISH AND FISHERY PRODUCTS

There is a growing interest in healthy foods made from bioactive ingredients to prevent diseases and improve nutrition in today's world population. For this reason, not only in science but also in industry, new electrohydrodynamic techniques have emerged. Electrospinning and electrospraying are promising techniques for the development of carrier materials because electrosprayed particles/electrospun fibers are easily fabricated and have appropriate physicochemical properties for the encapsulation of bioactive compounds in the production of various functional foods (Coelho et al., 2021).

Nanocapsulation of probiotic bacteria (*Lactobacillus rhamnosus*) could be evaluated as a natural/novel technique for inhibiting microbial growth in rainbow trout (*Onchorhyncus mykiss*) fish fillets (Ceylan et al, 2018). The fiber

films were used to preserve Japanese sea bass (*Lateolabrax japonicus*) during cold storage in the refrigerator. Poly(hexamethylene biguanide) hydrochloride (PHMB) fiber films effectively retarded the production of total volatile basic nitrogen (TVB-N), inhibited bacterial growth, and extended the shelf-life of Japanese sea bass (*Lateolabrax japonicus*) (Zhang et al., 2021). An electrospun film of collagen (Col)/zein (ZN) was encapsulated with different concentrations of gallic acid to preserve tilapia muscle (*Oreochromis niloticus*) (GA). The electrospun Col/ZN/GA films with 8% (w/w) gallic acid (GA8) extended the shelf-life of this fish muscle by at least two days, according to this study (Song et al., 2022). Electrospinning with low concentrations of citric acid, acetic acid, and malic acid in water in various solvents was used to create fish gelatin nanofilms. In water-based binary solvents, no nanofilms could be produced; however, quaternary and ternary solvents produced good results (Mahmood et al., 2019). Not only were gelatin-sodium alginate nanofibers used to protect live probiotics, but they were also used to extend the shelf-life of fresh silver carp *(Hypophthalmichthys molitrix)* fillets loaded with probiotics. During two weeks of cold storage in the refrigerator, they also inhibited the growth of foodborne pathogenic bacteria (*Vibrio parahaemolyticus*, *Salmonella Typhimurium*, *Listeria monocytogenes*, and *S. aureus*). Because the GE-SA nanofibers produced demonstrated acceptable resistance, the electrospinning technique was recommended as a brilliant method for use in heat-treated foods (Ghalehjooghi et al., 2022). A polylactic acid (PLA)-based antimicrobial film coated with mixed gelatin/chitosan nanofibers (GC-NF) spiked with 0.4% nisin preserved Asian sea bass (*Lates calcarifer*) slices for 12 days at 4 °C (Gulzar et al., 2022). Grape seed oil (gsN) electrospun with nanofibers (gsN) was found to be highly effective against microbiological inhibition and rapid lipid oxidation in raw rainbow trout (*Onchorhyncus mykiss*) fish samples stored at 4±1°C (Ceylan et al., 2021).

Saffron (*Crocus sativus* L.) extract (SE) was nanocapsulated with zein nanofibers (CNS) via electrospinning, and CNS loaded with SE (ZNLSE) was prepared as a nanocoating material. Furthermore, their use in the preservation of sea bass (*Dicentrarchus labrax*) fillets was investigated. This study's findings confirmed that ZNLSE has significant potential as a coating material for extending the shelf-life of fishery products like sea bass fillets (Najafi et al., 2022). Electrospinning technology was used to successfully fabricate Au-zein

based nanomats (AuZ-Nm). The current study found that the sensory properties of the control group samples changed rapidly (up to 50%). Furthermore, AuZ-Nm has been reported to be able to be incorporated into refrigerated stored foods (Çetinkaya et al., 2021). Aqueous fish gelatin electrospinning was discovered to be an alternative material to mammalian gelatin source (Kwak et al., 2017). The encapsulation of fish oil in poly(vinyl alcohol) (PVA) nanofibers was investigated using emulsion electrospinning. Fish protein hydrolysate (FPH) or whey protein isolate (WPI) and PVA concentration were found to have a positive effect on fiber morphology regardless of the emulsifier used (Garcia-Moreno et al., 2016). Microfibers were created using electrospinning technology from European fish eel (*Anguilla anguilla*) skin gelatin, and their ability to encapsulate European fish eel (*Anguilla anguilla*) oil (EO) was tested. The findings of this study indicated that EO-loaded microfibers could be used as effective encapsulation materials in nutraceutical and food applications (Taktak et al., 2021). Another study used electrospraying technology to encapsulate omega-3-rich fish oil. Various zein protein solutions were used to prepare electrospun zein fibers. Zein solutions were prepared in either 70% (w/w) aqueous ethanol/isopropanol or 30% (w/w) fish oil and then dispersed. The effects of polymer solution properties on electrospun fibers were successfully determined, as were the potential practical applications of these ultrathin zein fibers (Moomand & Lim, 2015). A protein-based halochromic electrospun nanosensor made of zein nanofibers and alizarin was developed to monitor the freshness of rainbow trout (*Onchorhynchus mykiss*) fillets. This electrospun nanosensor's color changes were found to be highly correlated with chemical and microbiological spoilage in trout fillets (Aghaei et al., 2020). Electrospinning was used to create nanomats containing nisin and curcumin, which increased the shelf-life of rainbow trout fillets (*Onchorhyncus mykiss*) during refrigerated storage (Meral et al., 2019). Biogenic amines, in particular, can form during the processing and storage of fermented foods. Food spoilage and food poisoning are caused by these compounds (Yerlikaya and Gökolu, 2002). Polyvinyl alcohol electrospun nanofibers (PAEN) with Ag nanoparticles were designed as nanosensors for detecting biogenic amines in shrimp meat (Marega et al., 2015).

6. CONCLUSION

Packaging techniques have evolved in response to consumer demands and desires. Among the methods for producing nanofibers, electrospinning stands out for its easy of use, as it is scalable, versatile, cost-effective and has a short processing time. Electrospun fibers have many advantages in terms of functionalities and desired properties for the food industry, making them a preferred material used in many food and fishery product packaging applications. These electrospun functional packaging materials have advantageous properties in that they can not only prevent microbiological and chemical spoilage of fishery and food products, but they can also extend the shelf-life of these products. Despite extensive research into electrospinning, it has been discovered that few studies have been conducted on the production of electrospinning nanofibers and the use of this technique for food and fishery products. As a result, this is a relatively new topic, with only a few studies on the use of electrospinning nanofibres for fish and fishery products. Furthermore, there is a growing interest in using electrospun nanofibers to encapsulate various bioactive food products. Further research into the use of electrospinning technology and its applications in food and fishery products should be conducted in the future.

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CHAPTER 2

SALINITY TOLERANCE AND PLASMA OSMOLARITY IN BONY FISHES

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1. INTRODUCTION

In fishes, excretion and osmoregulation systems are closely related and usually carried out by the kidneys and gills. Living cells need an environment that contains certain substances, including ions, dissolved in water with certain densities. The amounts of these substances determine the osmotic density of body fluids (Demir, 2006). Regulation of osmotic density is expressed as osmoregulation. Although the osmoregulation capacity varies according to the species, it is especially higher in diadromous fish species. The transition of fish between waters with different ion densities brings with some physiological adaptations. Salinity change causes various physiological adaptive responses in aquatic organisms such as disruption of electrolyte balance, stimulation of energy metabolism, and increase in stress-related hormones in plasma. (Liu et al., 2007). The most important of these responses that can be measured is osmoregulatory capacity. Depending upon the increase in salinity, the plasma osmolarity increases, thus keeping the osmotic pressure of the fish in balance. Adaptation of fish to different salinity values is very important especially in terms of creating species diversity in different biotopes of ornamental fishes. Adaptation of freshwater and especially brackish water species to salt water enables these species to be used in marine aquariums. Although aquarism is generally regarded as a hobby, it has actually turned into an agricultural sector that attracts millions of people around the world with has a very high economic return in terms of aquaculture (Hekimoğlu, 2006). Therefore, it is very important for the ornamental fisheries sector to ensure the efficient production of marine and fresh water ornamental fish species. The interest in marine-reef aquariums in our country and in the world has been increasing in recent years. However, the very limited production of marine fish species used in aquariums and the fact that they are procured directly from the natural area, makes these species more expensive than freshwater ornamental fish species and limits the interest in marine-reef aquariums. This situation leads to the idea of using fresh water and brackish water fish species with relatively high salinity tolerance, easy production and striking colors in marine aquariums. Approximately, 90% of freshwater ornamental fish species are supplied by aquaculture, while the remaining 10 % is obtained from nature by hunting. On the other hand, in marine aquariums, the situation is the opposite, about 95 % of which is

obtained by hunting and a very small part by aquaculture (Gopakumar and Ignatius, 2006). However, it is also important for fish introduction studies that freshwater, brackish water and saltwater fishes living in natural waters can be adapted to natural environments with different salinity levels.

Apart from osmolarity measurement in blood plasma, in the physiological evaluation of salinity tolerance of fishes, some tissues and organs related to the osmoregulation system of individuals exposed to different salinity levels are dissected and the Na^+-K^+ ATPase enzyme activities are examined.

Creating trials at different salinity levels

Determining the osmoregulatory capacity of fishes, glass aquariums or tanks made of PVC and various materials, which vary according to the fish species, are used. It is key factor that the species is taken to the trial environment at the appropriate stock density and that the water quality conditions in its natural life (such as; electrical conductivity, pH, temperature, hardness, dissolved oxygen) are provided. The salinity level being the only variable parameter throughout the experiment is essential in order to accurately determine the osmoregulation capacity. Additionally, in order to maintain the water quality throughout the experiment, it is necessary to change the water at certain rates and to use various internal filter elements (such as sponge filter, pipe filter) (Fig. 1).

Figure 1. Experiment setup and filtration of trial aquariums.

During the experiment, it would be appropriate to feed twice a day at a rate of 5% of the total body weight, according to Hekimoğlu and Alpbaz (2003). In order to evaluate the differences between the groups in the osmolarity and osmolality measurement values to be made in blood plasma and aquarium water, at least three repetitions of the experiment are required (Fig. 2). In addition, individuals of the same species to be used in the experiment should have close total length and weight values, and individuals of the same age who have reached sexual maturity should be used if possible. The study is carried out by increasing the number of trial groups according to the salinity levels determined in the study. The crucial point in studies on determining salinity tolerance is how many individuals the groups will consist of, at what rate the salinity will be increased and when the experiment will be terminated. However, it is also important point that the salt to be used in the experiment is isolated from the sea, does not contain any additives, non-iodized and is produced especially for use in marine aquariums.

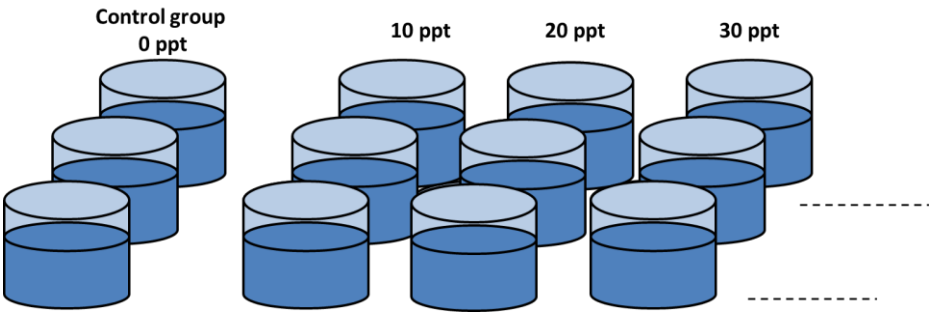

Figure 2. Schematic representation of an exemplary salinity tolerance determination study experimental setup.

There are differences in previous studies about the rate of periodic salinity increase. Naiman et al. (1976) continued the experiment with a total weekly salinity increase of ‰ 17.5 in the *Cyprinodon milleri* species, which spread in waters of ‰ 22 salinity. Perschbacher et al. (2011) reached a salinity level of ‰ 80 in *Fundulus grandis* in 20 days. This shows that the average daily salt increase is 4 ppt. In order to minimize the stress factor that will occur due to the increase in salinity, the lowest possible level of daily salt increase suggests that the adaptation will be healthier. The stocking ratios in the experimental groups were 12 individuals per aquarium in similar studies, but this can be evaluated by considering the characteristics of the species. It is important to determine the total number of individuals to be statistically sufficient for each salinity groups. Trial termination is usually accomplished by observing a high number of deaths at the highest salinity level. In some studies, the death of approximately 40 % of the number of individuals in the experiment after reaching the determined salinity levels (Nordlie et al., 1992) is accepted as the lethal effect of salinity and the work is terminated. However, the fish's cessation of feed intake may be seen as a sufficient response to termination of the trial. Because the cessation of bait intake shows that osmoregulation cannot occur in a healthy way and the fish will die in the coming days.

Blood serum uptake from fish samples

For small size species such as ornamental fishes, it is generally appropriate to use insulin injectors with 1 cc volume, 26 G x ½" needle tip or hematocrit capillary tubes with heparin. Thicker-tipped needles can be used for large size fishes and blood collection can be performed by cutting the tail stem, depending on the analysis to be performed in the study. However, it would be more appropriate to take the blood taken directly into the injector chamber without being taken out of the body in osmolarity measurements. Fish from which blood will be drawn should be anesthetized with 0.2 mL/L 2-Phenoxyethanol and the blood collection area should be thoroughly cleaned before the injection procedure. Physiological anticoagulant (5000 I.U./mL heparin sodium) should be taken into the syringe and contact with all surfaces of the syringe chamber should be ensured before each blood collection procedure in order to prevent blood coagulation. Depending on the amount of blood to be drawn, tubes containing EDTA may be used for higher volumes (Fig. 3).

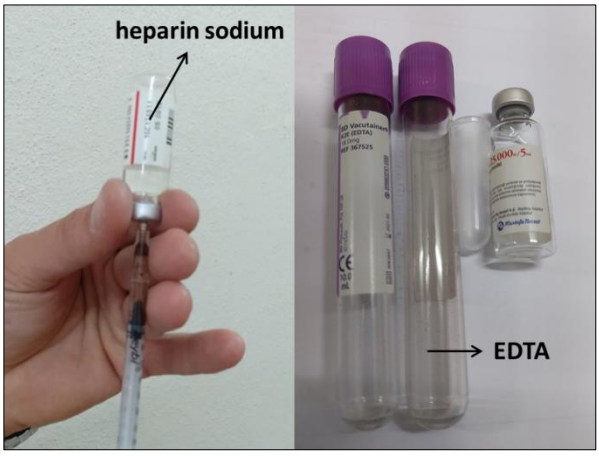

Figure 3. Heparin sodium solution and tubes containing EDTA used for anticoagulation.

Especially in ornamental fishes, blood is uptaken from the caudal vein (Fig. 4), close to the base of the tail, from the ventral part of the caudal stem and behind the anal opening (Dernekbaşı, 2012).

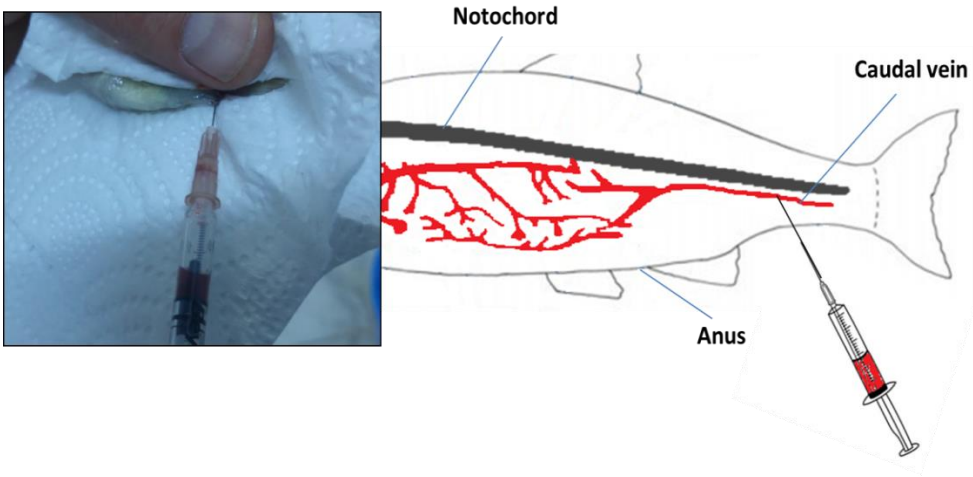

Figure 4. Blood collection from the caudal vein using an injector.

Separation of blood serum and measurement

Blood samples taken into microcentrifuge tubes are placed in appropriate devices and centrifuged at 1500 G for 5 minutes (Nordlie, 1987; Haney, 1999). The supernatant obtained after centrifugation is taken into a separate microcentrifuge tube (Fig. 5). If the blood serums obtained will not be measured immediately, they can be stored in a deep freezer at -80 ºC. The osmolarity values of the blood serum taken from the fish belonging to the groups are expressed in mOsm/kg or mOsmol/L. Osmolarity; It is the milli-osmol value of the substances that have an osmotic effect per liter of the liquid (blood serum) phase. Osmolality; It is the milli-osmol value of the substances acting in the liter of water (aquarium or tank water) in the liquid phase (Albayrak and San, 1976). In this context, osmolality measurements are made in the same devices by taking water samples from the aquariums where the blood serum is taken from the fish samples. Thus, by comparing the measurements of serum and water, the balancing of the osmotic pressure in the fish is interpreted.

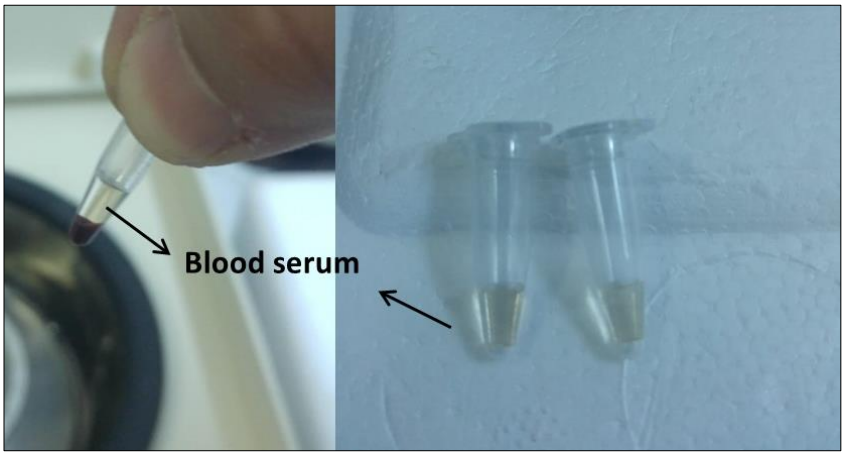

Figure 5. Separation of serum by centrifugation of blood samples.

It is important to homogenize the samples with a mini spin shaker for 1 minute before measuring in osmometer devices in order to obtain healthy results. Generally, osmometer devices with 20, 50 or 100 µL sample chambers and different measuring ranges are available. These devices generally work on the freezing/boiling point principle of liquids (Fig. 6). Samples placed in the chamber with an automatic pipette are measured in approximately 1 minute, depending on the model of the device. It is important to make measurements of the same sample at least twice in order to evaluate the deviations that may occur between the measurements.

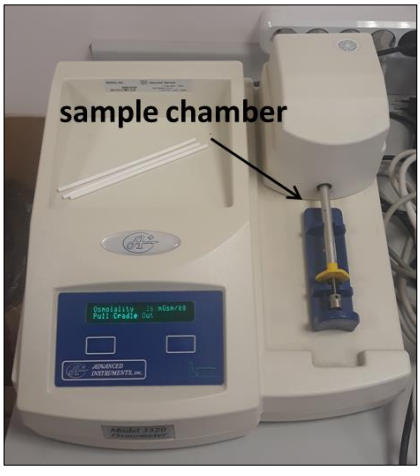

Figure 6. An example of an osmometer device.

2. CONCLUSION

There are some previous studies on the osmoregulation system and salinity tolerance in fishes. However, it is seen that natural fish species which are generally considered as food, are used in these studies. Studies on the salinity tolerance and osmoregulation of ornamental fishes are so limited. It can be thought that this is due to reasons such as the smaller size species of ornamental fish, the difficulty of blood collection and the inability to obtain sufficient volumes of blood serum. However, problems related to serum volume can be overcome by measuring the obtained blood serum by diluting it with distilled water. The limited production of marine aquarium fish provides an alternative with the adaptation of brackish water species to salt water. Brackish water fish species such as *Monodactylus argenteus*, *Scatophagus argus*, *Scatophagus tetracanthus* among ornamental fish (FishBase, 2022) have been successfully evaluated in marine-reef aquariums for many years. It is very important to add new species with high osmoregulatory capacity to these species. When the previous literature on salinity tolerance of ornamental fishes is examined, it is generally seen that Cyprinodontidae (Lotan, 1971; Naiman et al., 1976; Nordlie, 1987; Haney, 1999; Perschbacher, et al., 2011; Sepil, 2020) and Poeciliidae species (Nordlie, et al., 1992) are used. Adding species belonging to new families to these studies will enrich the scientific infrastructure on this subject. However, fish that have reached sexual maturity are generally used in these studies. Evaluation of the effects of increased salinity on fecundity, egg hatching and larval development, especially determining the salinity tolerance of larvae by methods such as Na^+-K^+ ATPase enzyme activity and chloride cells antibody markings will increase the knowledge on this subject.

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CHAPTER 3

MICROBIOLOGICAL CHARACTERISTICS OF SOME BACTERIAL AGENTS ISOLATED FROM RAINBOW TROUT FARMS

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1. INTRODUCTION

Aquaculture is a major industry that meets a significant portion of the world's food requirements and is reported by the Food and Agriculture Organisation (FAO) as the fastest growing food sector in the world. Aquaculture accounts for approximately 30% of world fisheries production (Davenport et al., 2003).

In addition to water quality, suitable environmental conditions, feed supply, marketing and labour force problems, the most important problem causing economic losses in freshwater and marine aquaculture in our country as in the whole world is the presence of various diseases (Timur and Timur, 2003).

Bacterial disease agents account for 36% of economic losses in aquaculture (Çağırgan, 2007). The first of the steps targeted for the treatment of bacterial diseases is the isolation and identification of the agent. While enriched and specific media are used for the isolation of the agent, identification is carried out by phenotypic, serotypic and genotypic methods (Önalan, 2016).

In addition to water quality, suitable environmental conditions, feed supply, marketing and labour force problems in aquaculture, the most important problem causing economic losses in freshwater and marine aquaculture in our country as in the whole world is the presence of various diseases (Timur and Timur, 2003).

The most common bacterial diseases in our country are *Yersinia ruckeri*, *Lactococcus garvieae*, *Vibrio salmonicida*, *Aeromonas hydrophila*, *Listonella anguillarum*, *Pseudomonas picicida*, *Flavobacterium psychophilium*, *Renibacterium salmoninarium* in freshwater fish. In marine fish; *Listonella anguillarum* (Vibriosis), *Photobacterium damsela subsp. Pi*scidia (Pasteurellosis), *Tenacibaculum maritimum* (Myxobacteriosis), *Streptococcus iniae*, *Lactococcus garvieae*, *Streptococcus uberis* (Streptococcosis, Lactococcosis), *Aeromonas hydrophila* (Motile Aeromonas Septicaemia) and *Pseudomonas anguilliseptica* (Winter diseases) (Timur and Timur, 1985).

If we look at the general structure of bacteria; they have a "prokaryotic" cell structure because they do not have nuclei and membrane-enclosed organelles. Molecules such as chlorophyll and oxygenated respiratory enzymes are found on the folds extending from the cell membrane to the cytoplasm or free in the cytoplasm (Anonymous-a, 2018).

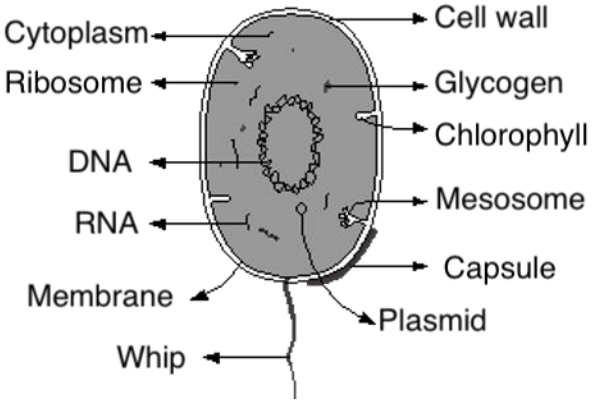

Figure 1. General cell structure of bacteria (Anonymous-a, 2018)

All bacterial cells contain membrane, wall, ribosome, DNA, RNA and various enzyme systems. In some species, in addition to these structures, there may be some specialised functions. Cilia and flagella, which extend outward from the cell membrane, provide movement and protection. In very few species, there is a third cell covering. This structure, called capsule, increases the resistance to unfavourable conditions. For this reason, encapsulated bacteria are usually pathogenic (disease-causing). The cell wall in bacteria is made of protein, fat and carbohydrate and does not contain cellulose. It shapes and protects the bacteria (Anonymous-a, 2018).

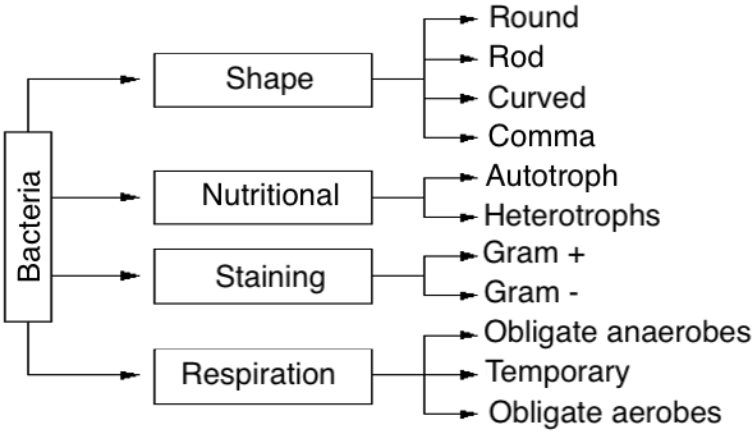

Figure 2. General classification of bacteria (Anonymous-a, 2018)

DNA replication and synthesis process in bacteria synthesises the complement of each complement of the double helix of DNA separately from the origin to two directions. It has been proven by auto radiographic experiments that synthesis in bacterial cells starts from the origin and continues in two directions (Anonymous-a, 2018).

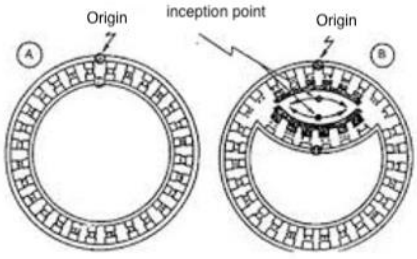

Figure 3. DNA synthesis in bacteria continues in a circular manner. A) Circular double-stranded DNA chromosome B) DNA synthesis continuing in both directions at the point of origin (Arslan, 2018).

Lake Van basin has great importance in terms of natural water resources and endemic fish species. In this respect, it is necessary to carry out continuous and sustainable inspections of the enterprises that both breed and sell fish to neighbouring enterprises and provinces in terms of diseases. Some studies conducted in Lake Van basin in terms of bacterial diseases in the field of aquaculture are specific to the factors. In this study, different colonies of bacteria isolated from fish samples collected from fish farms in Van province, where rainbow trout farming is carried out and fish farms that grow and sell their own fish are selected, will be examined microbiologically. We think that the results of the study will have an economic contribution to all institutions and organisations engaged in fish farming and aquaculture in our country. The study constitutes the microbiological part of a project. In the continuation of the project, molecular characterisation and typing stages were carried out.

2. MATERIAL AND METHOD

Fish Material and Sampling

The rainbow trout (*Oncorhynchus mykiss*) used in the study were selected from the farms registered in the Ministry of Food, Agriculture and Livestock in Van province, especially the farms that raise fry and sell to

neighbouring farms and provinces (Table 1). The farms were visited in October, May and August for sampling. Eight symptomatic fish were purchased from each rainbow trout farm. Age, weight and sex were not discriminated.

Tablo 1. Rainbow trout farms to be sampled

No	Farms name	District Located in	offspring production
1	Buzlupınar	Çatak	✓
2	Yeşilsu	Çatak	✓
3	Elfa	Çatak	✓
4	Şifa	Gürpınar	✓
5	Özçatak	Çatak	✓
6	Bayazsu	Çatak	✓

A transport container (Rubbermaid) with a volume of 45 litres was used for sampling. The transport container (Rubbermaid) to be used for the study was cleaned with 70% ethanol before sampling, ice sponges (Thermo) were placed inside and kept overnight at -20 ^{o}C with the lid open. Before the field study, a thermometer with probe was placed in the transport container (Rubbermaid) and the temperature was controlled during sampling. The samples taken from each facility were placed in locked refrigerator bags and brought to the laboratory on the same day in the transport container (Rubbermaid). During sampling, fish showing symptoms such as swimming disorder, unilateral or bilateral exophthalmos in the eyes, blackening in the dorsal region, circular haemorrhage around the eye and immobility in the water were selected.

Method

Bacteria Isolation

It was carried out at Van Yüzüncü Yıl University, Faculty of Fisheries, and Disease Laboratory. Fish samples were cleaned with 70% ethyl alcohol to prevent contamination (Önalan and Arabacı, 2020).

The rainbow trout specimens used in the study were cultured on Tryptic Soya Agar (TSA) medium from the anterior kidneys, symptomatic fin tissues and basractan. The inoculated media were incubated at 15, 21, 26 and 37 °C for 48 hours. After the incubation period, the colonies formed by the bacteria were examined in terms of morphological characteristics such as colour, shape and

brightness. The growing bacteria in each different colony structure were purified by re-cultivation separately (Önalan and Çevik, 2020).

Determination of phenotypic characterisation of bacteria

For this purpose, catalase, oxidase, motility, Gram staining and API kits were used to characterise and classify the isolated bacteria in terms of biochemical properties (Önalan, 2019).

Movement Test

Each isolated bacterial colony sample was placed on a slide, 1 drop of 0.6% FTS was added on it and then examined under a binocular microscope (Leica ICC50 HD) with magnifications of 4X, 10X, 40X and 100X in terms of negative examination. During microscopic examination; active or passive movements, morphological structures and viability of the bacteria were examined (Çağırgan, 2007).

Gram Staining

In order to perform gram staining for each different bacterial colony isolated, 1 drop of 0.6% FTS was added on the slide from the pure bacteria grown as a result of the incubation period and the mixed bacterial suspension was fixed by passing the flame 3 times. Then, the staining process was completed by treatment with crystal violet for 1 min, lugol for 1 min, alcohol for 15 s and safranin for 1 min. The dried preparations were examined under a binocular microscope (Leica ICC50 HD) with magnifications of 4X, 10X, 40X and 100X, respectively. Isolates with blue-purple colour after Gram staining were evaluated as Gr (+) and isolates with red-pink colour were evaluated as Gr (-) (Önalan, 2019).

Oxidase Test

Bactident Oxidase commercial kit was used for oxidase test. After the incubation period, a small amount of each different colony was taken with the help of a sterile extract and applied to sterile filter papers soaked with oxidase kit. Within 30 seconds, the colour changes on the paper surface were examined. The formation of blue colour was evaluated as oxidase positive, while the absence of any colour was considered as a negative result (Çağırgan, 2007).

Catalase Test

For the catalase test, 1-2 drops of 3% hydrogen peroxide were dropped onto the slide and colonies with different morphological characteristics were mixed with a sterile core. Bacteria that formed gas on the slide due to the release of oxygen were considered as catalase positive, and bacteria without any gas formation were considered as catalase negative (Austin and Austin, 1987).

Determination of biochemical properties of bacteria

After morphological characteristics and catalase, oxidase, gram staining results, bacteria were separated according to these results and bacterial suspension solutions were loaded into API kits. The incubation period was 18 hours at 37 ^{o}C for both bacterial growth and response to chemicals. At the end of 18 hours, bacteria were evaluated in terms of 46 different biochemical tests and identification procedures were carried out according to the percentage ratio (Önalan and Çevik, 2020).

3. FINDINGS

Field Data Obtained During Sampling

It was observed that the water temperature varied between 12-16 ^{o}C during the collection of samples from the farms producing and selling fish in Van province. It was observed that the farms were not suitable in terms of tools and equipment in fish farms, but the tools and equipment were complete in large-scale production enterprises. In addition, it was observed that aquaculture engineers were working in the farms and the number of aquaculture engineers varied according to the fish capacity.

During the sampling, fishes with colour darkening, immobility, separate and stationary fishes, whiteness on dorsal fins and exophthalmos were sampled (Figure 4).

Figure 4. Symptomatic fish samples from which bacteria were isolated in the study

Bacteria Isolations

Fish samples taken from the field were brought to the laboratory on the same day (within maximum 3 hours) at +4 ºC. After necropsy of fish samples from symptomatic external surfaces and aseptic conditions, bacterial cultures from kidney, spleen, intestine tissue and symptomatic organs were sown on TSA medium. Pictures of some fish samples necropsied for cultivation are given below (Figure 5).

Figure 5. Bacterial cultivation areas of symptomatic fish samples after necropsy

After a 24-hour incubation period at 37 ºC in TSA medium, different types of colonies were re-cultured and different colonies were purified separately.

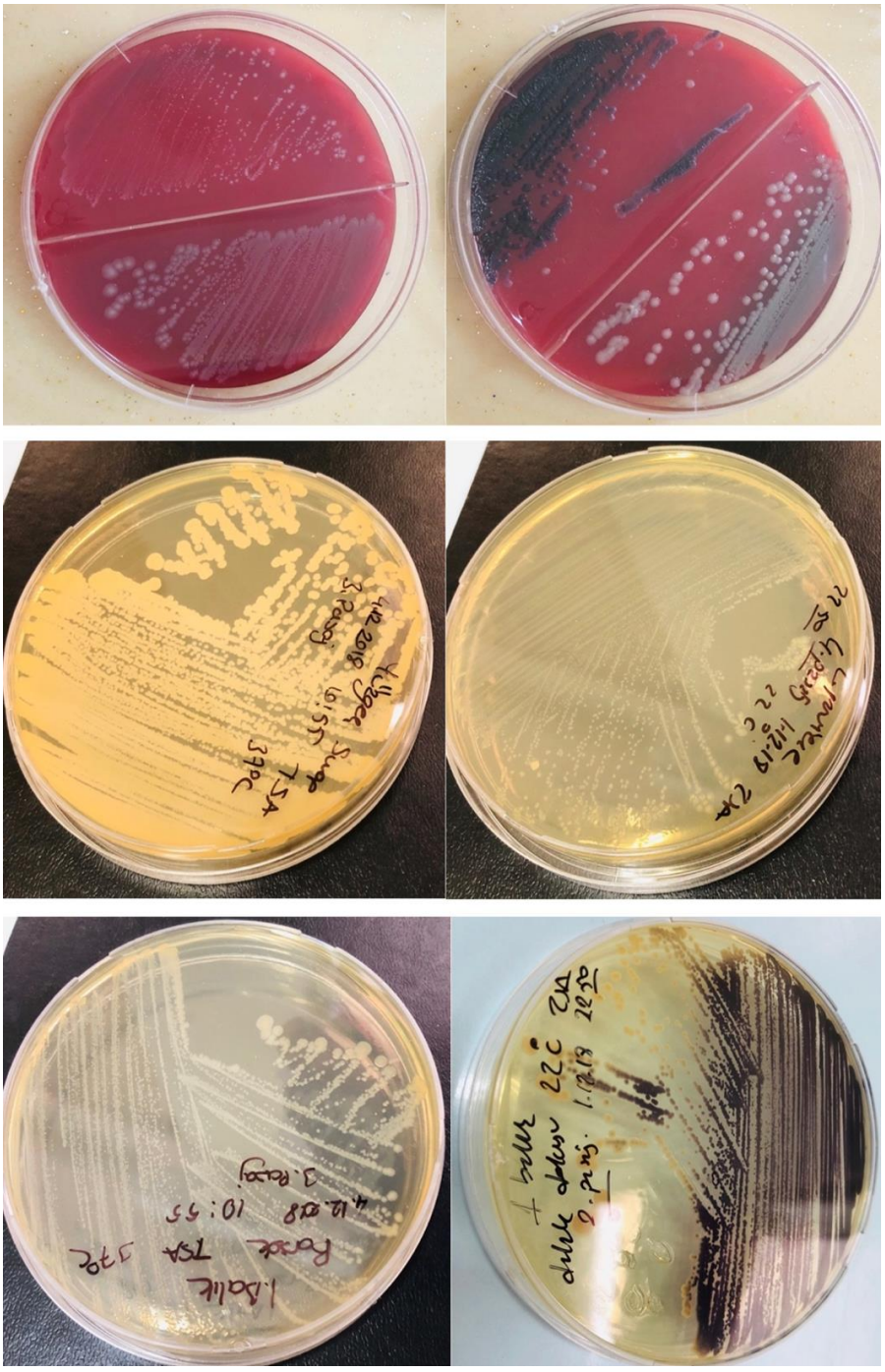

Figure 6. Colonies developing on the media after bacterial cultivation

Bacteria purified on solid medium were cultured in TSB liquid medium (85% TSB + 15% Glycerol) for both phenotypic and molecular steps.

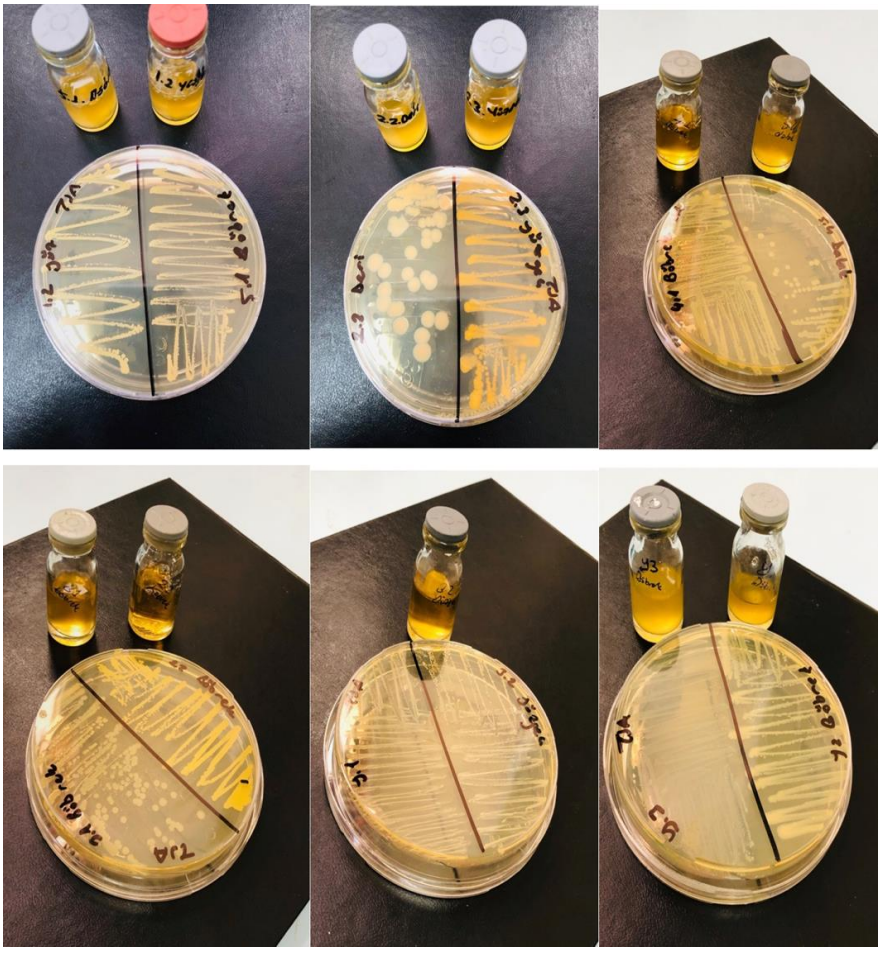

Figure 7. Bacteria growing on solid and liquid media

Determination of biochemical properties

API kits were also used to determine the phenotypic characterisation of the purely isolated bacteria. The application and evaluation of the results were carried out in accordance with the instructions of the manufacturer (Biomerioux). The biochemical test results of the bacterial isolates isolated in the study are given below (Table 3).

Table 3. Some phenotypic characteristics of isolated bacteria

	1-S. epidermidis (API ID32Sta)	2-B. subtilis (API Coryne)	5-S.epidermidis (APIID32Sta)	10- A. Salmonicida (API 20E)	11- S. warneri (API ID32Stap)	12- S. warneri (API ID32Stap)	15- B. Thuringiensis (API Coryne)	16- Micrococcus luteus	L. garvieae (API 32Strep)	L. garvieae (API32 Strep)	L. anguillarum (API 20E)
CAT		+							-	-	
OX				+					-	-	+
OPNG				-							+
ADH	+		+	+	+	+			+	+	+
LDC (Lys)				-							-
ODC	-		-	-	-	-					-
CIT				-							+
H_2S				-							-
URE	+	+	+	-	+	+			-	-	-
TDA (Trp)				-							-
IND				-							+
VP	+		+	-	+	+			+	+	+
GEL		+		+							+
GLU	+	+	+	+	+	+			+	+	+
MAN	-	+	-	+	+	+			+	+	+
INO				-							-
SOR				-					-	-	+
RHA				-							-
SAC	+	+	+	-	+	+			-	-	+
MEL				-					-	-	-
AMY				-							+
ARA	-		-	-	-	-					-
bGAR									-	-	
bGUR	-	-	-		+	+			-	-	
aGAL									-	-	
PAL	-	+	-		-	-			-	-	
RIB	-	+	-		-	-			-	-	
LAC	+	-	+		-	-			-	-	
TRE	-		-		+	+			+	+	
RAF	-		-		-	-			-	-	
LARA									-	-	
DARL									-	-	
CDEX									-	-	
APPA									+	+	
bGAL	-	+	-		-	-			-	-	
PyrA	-	+	-		-	-			+	+	
bNAG	-	-	-		-	-			+	+	
GTA									-	-	
HIP									-	-	
GLYG									-	-	
PUL									-	-	
MAL	+	+	+		+	+			+	+	
MLZ									-	-	
TAG									+	+	
bMAN									-	-	
FRU	+		+	+	+	+					
ESC	-	+	-	-							
MNE	-		-		-	-					
CEL	-		-		-	-					

* Names of the isolates coded in the table; 1- *Staphylococcus epidermidis*, 2- *Bacillus subtilis*, 5- *Staphylococcus epidermidis*, 10- *Aeromonas salmonicida*, 11- *Staphylococcus warneri*, 12- *Staphylococcus warneri*, 15- *Bacillus thuringiensis*, 16- *Micrococcus luteus*, 17- *Lactococcus garvieae*, 18- *Lactococcus garvieae*, 19- *Listonella anguillarum*

4. DISCUSSION AND CONCLUSION

Rainbow trout, which is the most cultivated fish in the world, is preferred over other salmonid fish because of its short incubation period, easy adaptation to environmental conditions, high ability to utilise natural and artificial feed and resistance to diseases (Kum et al., 2004).

In recent years, rainbow trout production in Turkey has exceeded 100,000 tonnes. With this progress achieved in a short time, entrepreneurs have faced many problems. In rainbow trout farming, problems such as the lack of gene source in our country, brood stock and management, certification, hatchery, diseases and marketing are the main problems challenging trout farming (Arabacı, 2007).

Bacterial diseases have an important place among disease problems and gram positive cocci have been identified as important fish pathogens in the last decade. Many epidemic and sporadic cases caused by gram positive pathogens have been reported in various parts of the world (İspir et al., 2013).

Rainbow trout (*Oncorhynchus mykiss*) is a North American origin fish belonging to Oncorhynchus genus of Salmonidae family. The body of salmonid fish is elongated and has a rayless adipose fin between the rayed dorsal fin and caudal fin. The dorsal fin has 10-12 soft rays and the anal fin has 8-12 soft rays. Scales are cycloid and small. Lateral line is covered with 100 to 150 scales, complete, slightly anteriorly. Whiskers are absent. Scales are small. The upper part and back of the head are steel blue, blue-green, yellow-green and almost brown. Body margins silvery, white or pale yellow-green to grey. The abdomen is silvery white or yellow. There is also a blurred pink, bluish or broad light pink band and numerous small spots on the body margins. At the time of spawning in brood stock the colour becomes very dark and the lateral line very red. These fish reach a maximum length of 70 cm and a weight of 7 kg in Europe (Funke and Kissling, 2004).

In rainbow trout, *Aeromonas salmonicida*, *Renibacterium salmoninarum*, *Flavobacterium psychrophilum*, *Yersinia ruckeri*, *Listonella anguillarum*, *Pseudomonas fluorescens*, *Vibrio salmonicida* and *Lactococcus garvieae* (Balta et al., 2016).

Listonellosis is one of the most important bacterial infections caused by *Listonella anguillarum* and is reported as a cause of disease in freshwater fish (Uluköy et al., 2013). *Vibrio anguillarum*, which is defined as the specific agent

of the disease, is one of the species in the genus vibrio in the family Vibrinocea. The causative agent is usually flat or comma-shaped, gram-negative, motile, non-spore, non-encapsulated, aerobic or facultative anaerobic (Arda et al., 2002). Vibrio infection can spread rapidly when fish are intensively stocked and can cause morbidity up to 100% in facilities where the infection spreads in for-profit systems and significant mortality exceeding 50% when outbreaks progress. *Listonella anguillarum* and closely related bacterial species are commonly found in estuaries, coastal harbour habitats and can be easily isolated from different environmental sources (Austin and Austin, 1987).

RTFS is an infection caused by *Flavobacterium psychrophilum* and was first described by Davis in 1946 when typical lesions were observed around the peduncle in rainbow trout. For this reason, it was named "Peduncle disease". The disease is called "Cold Water Disease" because it occurs mostly in cold waters of 10 °C, "If Disease" because of the ulcerative lesion in the shape of a saddle on the back, "Fry mortality syndrome" because it causes death in fry and "Rainbow Trout Fry Mortality Syndrome, (RTFS)" because it is seen in rainbow trout. *Flavobacterium psychrophilum*, the causative agent of the disease, was initially named *Cytophaga psychrophila* and then *Flexibacter psychrophilus*, but it was concluded that it would be more appropriate to be called *Flavobacterium psychrophilum* considering DNA-rRNA hybridisation data (Çağırgan, 2007).

Lactococcosis caused by *Lactococcus garvieae* is a septicaemic disease that causes economic losses in freshwater and marine fish farmed in many countries of the world when water temperature increases in summer months (Çağırgan, 2004). Species belonging to the genus Lactococcus are included in the family Streptococcaceae. Streptococcosis in rainbow trout was first reported by Hoshina in Japan in 1958. It was first observed in a small family farm in Karacasu district of Aydın in September 1992. In the same year, 5 different epizootics were observed in the enterprise. In 1993, when the water temperature was 12 °C, it was isolated from the intestines of completely healthy fish and when the water temperature increased to 15 °C, it caused 60% mortality within 3 days. When the water temperature increased to 15 °C and above in 2000, lactococcosis was observed in other trout farms and the pathogen spread to almost all trout farms by fish transfers and caused serious losses. It is still one of the most important fish pathogens threatening trout culture (Çağırgan, 2007).

Bacterial haemorrhagic septicaemia infection is caused by motile Aeromonas species which are commonly found in water and cause septicaemia infections in fish and cold-blooded animals and enteric, septicaemia or local infections in various animal species and humans (Burka et al., 1997). *A. hydrophila* causes irregular haemorrhages on the body surface, exophthalmos, darkening of the skin colour, erosion, ulcers, inflammation, bloody ascites in the abdominal cavity, invagination in the intestines, haemorrhage around the anus, hyaline degeneration in the muscle layer of the stomach and intestines and vacuolation in muscle cells, haemorrhage in the submucosa, oedema; hyperaemia, haemorrhage, degeneration, necrosis and vacuoles in cells, inflammation in pancreas, lung, liver, kidney, gills and skin; atrophy and necrosis in the pancreas, haemorrhage and hemosiderin accumulation in the liver, hyperaemia, haemorrhage and vacuolation in the epithelium in the gills, adhesion in the gill lamellae, sub epithelial oedema and aneurism in the secondary lamellae (Çağırgan, 2007).

The bacteria isolated and purified in the study were preliminarily identified morphologically and microbiologically. For this purpose, Gram staining, catalase oxidase API kits were used for identification. As a different opinion regarding the phenotypic method used in the study, it has been reported by some researchers that API test kits are more suitable for rapid identification of human pathogens and that these kits give erroneous results when used for fish pathogens, especially because the incubation temperatures are not suitable for the incubation temperatures of fish pathogens (Balta and Balta Dengiz, 2017). It has been reported by many researchers that the agent is easily identified by using rapid diagnostic kits such as API 20E (Austin and Austin, 2017). Apart from API kits, automatic identification methods such as BD phonex ID and Vitek II are automatic system devices that can obtain more reliable results. However, the methods for these devices are carried out at high economic costs. In addition, the kits do not work with a single sample and we believe that they are useful in routine in areas with multiple samples.

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CHAPTER 4

THE PREDICTIVE MODELS USED FOR BACTERIAL GROWTH AND SHELF-LIFE OF FISHERY PRODUCTS DURING STORAGE

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1. INTRODUCTION

Pathogen microorganisms can contaminate foods, posing health risks when consumed. Foods contain not only pathogens, but also spoilage microorganisms found in nature and the environment. (Stavropoulou and Bezirtzoglou, 2019). Microorganisms from catching waters have the potential to contaminate fishery products. These products can quickly deteriorate after capture. Many factors, such as catching, distribution, storage, and processing influence the quality of these products. As a result, avoiding changes in food product parameters such as salt or water activity, temperature, pH and storage atmosphere among others, is very critical for preventing spoilage. (Garcia et al., 2022). To ensure food safety, the growth of pathogenic bacteria must be either destroyed or prevented. Many technologies have been discovered that inhibit the growth of these microorganisms. One of the most important of these methods is to keep food products cold or chilled temperatures (Ünlütürk and Turantaş, 1993). Because chilled foods have relatively short shelf lives, even when refrigerated, consumers generally believe they are "fresh," "healthy" and "wholesome". Shelf-life of food product is concerned with safety and consistency of quality. In another words, it is the period of time that a food product remains safe, protects its desired chemical, sensory, physical, microbiological, and functional characteristics. Morever, if stored under the recommended refrigerated conditions, it complies with any nutrition data label declaration. (Man, 2008). Therefore, to ensure safe and quality seafood consumption, a tracking system for the detection of pathogenic bacteria species should be developed at all stages of fishery products, from fishing to consumption (Kılınç, 2020).

In recent years, for predicting the bacterial growth on foods and for estimating the shelf-life of foods, the mathematical predictive models have been used in an increasing rate. These models have many advantages for example, the method employs the theory of suitable experimental design to hugely data of information while reducing the number of experiments. The showing growth model for specific spoilage microorganisms in fish not only gives has effectively informative to predict the quality of sensory indexes in different temperature profiles that change over time, but also includes quantitative data on the uncertainty caused by fish variability (Garcia et al., 2015).

Mathematical modelling is a significantly very important tool for determining between the time and temperature relationship in very perishable seafood products. It is very essential to observe the growth kinetics of different microorganism species in shelf-life predictions. When improving the shelf-life estimation models in seafood products, various models should be applied based on the microorganisms and the products (Genç and Diler, 2017). Analysis of home storage time distributions and domestic refrigerator temperatures for food safety risk assessment and shelf-life studies were studied by (Raccato et al., 2017). Predictive microbiology not only serves as a valuable tool to aid in the evaluation of the exposure phase 'quantitative microbiological risk assessment,' but these predictive models also give rise to changes in bacterial activity on food products from the moment of production or harvest to the moment of consumption, which can be predicted with variations in the parameters of food products (Rose et al., 2000). In contrast, these predictive models have some disadvantages and shortcomings, such as the fact that the results of predictive models were reported to be dissimilar to the results of real-world studies. In some cases, the models appeared to be 'fail or safe,' in the sense that the predicted growth rate or time to toxicity was faster or shorter than what actually occurred in the food, whereas in others, the predictions indicated that the product was safe when it was not. (Hytiia et al., 1999).

In this review article, the mathematical predictive models, growing bacteria groups in fishery products, using these models to predict bacterial growth and shelf-life of fishery products during storage were all explained. Furthermore, not only were the studies on developing predictive models to assume the shelf-life of raw and processed fishery products, but also some of the benefits and drawbacks of current modeling approaches were highlighted.

2. THE PREDICTIVE MODELS

A mathematical model is a description of a system using mathematical concepts and language. Mathematical modeling, on the other hand, is the process of trying to express mathematically or non-mathematically an event, phenomenon and the relationships between events, and revealing mathematical patterns within these events and phenomena (Tutak & Güder, 2014).

Similarly, the changes that will occur on microorganisms due to the effect of environmental factors can be determined by mathematical methods. Since the responses of microorganisms to environmental factors are reproducible, it is possible to characterize the responses of microorganisms in similar or different environments in terms of environmental factors affecting microbial growth and survival and to predict based on past observations (Jagannath, 2003).

With the science of predictive microbiology, which is a combination of the fields of mathematics and microbiology, microbial responses in different environmental conditions can be examined, and the effects of processing, distribution and storage processes on food quality and microbiological safety can be evaluated. Predictive microbiology, which is still new in practice, has an old history. The scientifically predictive model was first introduced to the industry by using it to determine the heat treatment required to destroy 1012 Clostridium botulinum type A spores (McMeekin et al., 2002; Jagannath, 2003).

Later, many predictive models have been developed according to their intended use. Today, predictive models can be classified as kinetic and probability models based on the microbiological event examined, empirical and mechanical models according to the modeling approach, and primary, secondary and tertiary models according to independent variables (Jagannath, 2003).

Primary Models

These models measure the microorganism's response over time with respect to a single condition. According to this condition, the growth curve of the microorganism can be defined. The growth curves of microorganisms basically contain 4 phases.

The main purpose of primary models is to describe the growth curves that show the behavior of microorganisms in food (Jaiswal and Jaiswal 2014). The growth curves of microorganisms mainly include 4 phases and the first 3 phases of the growth curve have a sigmoidal shape. In primary models, this sigmoidal structure of microorganisms can be explained by sigmoidal functions such as Monod, Gompertz, Logistic, Richards, Schunte, Stannard, and Baranyi (Zwietering, 1990).

Table 1. Commonly Used Primary Predictive Models

Primary Models	Equations
Logistic	$y = \frac{a}{[1 + \exp(\mathrm{b} - \mathrm{ct})]}$
Gompertz	$y = a\exp[-exp(\mathrm{b} - \mathrm{ct})]$
Richards	$y = a\{1 + v.exp[k(\tau - t)]\}^{\left(-\frac{1}{v}\right)}$
Stannard	$y = \mathrm{a}\left\{1 + \exp\left[-\frac{(l + kt)}{p}\right]\right\}^{(-p)}$
Schnute	$y = \left\{y_1^b + (y_2^b - y_1^b).\frac{1 - \exp[-a(t - \tau_1)]}{1 - \exp[-a(\tau_2 - \tau_1)]}\right\}^{1/b}$

Zwietering (1990)

In terms of the models, t: time, a, b, c, k and τ define the model parameters.

Secondary Models

Secondary models are models that can identify all biotic and abiotic parameters that can change microbial kinetics, such as water activity, temperature, pH factors (Stavropoulou et al., 2019). Secondary models in predictive microbiology are not as diverse as primary models, but the most common ones are the Arrhenius equation and the Square Root-Type Models (Pérez-Rodríguez 2014).

Ratkowski et al. (1982) found that the fit of Arrhenius plots, which is the expression of the absolute temperature versus logarithmic growth rate constant, to the aforementioned data was insufficient in describing bacterial growth in chemical reactions. Accordingly, they suggested that there was a linear relationship between the square root of the growth rate constant (r) and the absolute temperature (*T*) in degrees Kelvin as follows.

$$\sqrt{r} = \mathrm{b}(T - T_{min})$$

At higher temperatures, this linear relationship did not appear to adequately explain growth due to inactivation or denaturation of proteins or other factors. Therefore, the following nonlinear regression model has been

proposed to describe bacterial growth over the entire temperature range (Ratkowsky et al., 1983).

$$\sqrt{r} = \mathrm{b}(T - T_{min})\,\{1 - exp[c(T - T_{max})]\}$$

Table 2. Commonly Used Secondary Predictive Models

Secondary Models	Equations
Arrhenius	$\ln\left(\frac{N}{N_0}\right) = kt = k_0 \exp\left(\frac{-E_A}{RT}\right) t$
Ratkowsky et al., (1982)	$\sqrt{r} = \mathrm{b}(T - T_{min})$
Ratkowsky et al., (1983)	$\sqrt{r} = \mathrm{b}(T - T_{min})\,\{1 - exp[c(T - T_{max})]\}$

The parameters in the model are respectively N: number of bacteria, N_0: number of bacteria at the beginning, y: growth as $\ln(N/N_0)$, k: reaction rate constant, R: Gas constant (8.314 J/(Kmol), Ea: Activation energy, t: time, r: growth rate, b: regression coefficient constant, T: temperature (°C), Tmin: a hypothetical temperature (°C) depending on the characteristics of the organism, and Tmax: maximum temperature. Tmin can be obtained by linear regression relationship between the square root of maximum growth rate and temperature.

Tertiary Models

Tertiary models, on the other hand, include applications of one or more secondary models, in which algorithms are included in the calculation of variable conditions, using computer programs (Jaiswal and Jaiswal 2014). These models are combined primary and secondary models incorporated into computer applications that widely used in the food industry and research for microbiological prediction (Stavropoulou et al., 2019). Some software's and free download links for tertiary models are given in Table 5. For the application, the fit of data can be achieved with the iteration processes related to the determination of the initial values for the parameters in the model.

Table 3. The Commonly Used Software's and Access Links For Tertiary Predictive Models

Tertiary Model Software	Access Link
Baseline 1.0	www.baselineapp.com
ComBase	www.combase.cc
DMFit	http://www.ifr.ac.uk/safety/dmfit/
Unified Growth Prediction Model (UGPM)	http://www.aua.gr.psomas
GInaFit (Add-in for Microsoft excel)	www.cit.kuleuven.be/biotec/downloads.php
MicroHibro	www.microhibro.com
Food Spoilage and Safety Predictor (FSSP)	www.fssp.food.dtu.dk

Olaonipekun (2017)

Comparison of Models

Testing the accuracy of the model, in other words, determining how well the model fits the data is more important than applying the model. For this purpose, coefficient of determination (R^2) can be used to evaluate or compare models. The coefficient of determination ranges from $0 < R^2 < 1$, and its high value indicates that the predictive power of the model is good. The coefficient of determination with SS: Regression sum of square and RSS: Residual sum of square, is calculated as follows:

$$R^2 = \frac{SS}{RSS + SS}$$

It has been determined that predictive models generally give lower sum of squares (RSS) error as the number of parameters increases. Since the predictive models have different number of parameters, the sum of the squares of the error of the model will not provide sufficient information. Therefore, when comparing predictive models, it is recommended to consider the Adjusted R^2 value, which also takes into account the number of parameters in the model, instead of R^2. R^2: coefficient of determination, k: number of parameters, and n: number of observations, the corrected coefficient of determination is calculated as follows.

$$R^2_{adj} = 1 - \left[(1 - r^2)\left(\frac{n-1}{n-k-1}\right)\right]$$

Moreover, mean square error (MSE) or Root mean square error (RMSE) statistics are commonly used to evaluate the difference between estimated and observed measurements. The model with a lower MSE or RMSE (0 < MSE/RMSE) is accepted more precise (Brocklehurst, 2003). MSE and RMSE can be defined as below equations, where the p: number of parameters and n: number of observations.

$$MSE = \frac{\sum(Predicted - Observed)^2}{n-p}$$

$$MSE = \sqrt{\frac{\sum(Predicted - Observed)^2}{n-p}}$$

Bias and accuracy factors are also used as good fit criteria in model comparison. The accuracy factor shows the average deviation between model predicted and observed values, and the higher accuracy factor shows the lower precision. The bias factor is also used to denote the structural deviations of a model. When the bias factor is greater than>1, it points as the fail-safe model. The accuracy and bias factor can be defined as following expression respectively (Jaiswal and Jaiswal, 2014):

$$Accuracy\ factor = 10^{\ \frac{\sum log(\frac{Predicted}{Observed})}{n}}$$

$$Bias\ factor = 10^{\ \frac{\sum log(\frac{Observed}{Predicted})}{n}}$$

3. GROWING BACTERIA GROUPS IN FISHERY PRODUCTS

Fresh fish, particularly fatty species, are extremely perishable owing to oxidative deterioration of fish flesh and an increased microbial load on the fish (Giannokourou et al., 2022). In modified atmosphere packaged (MAP)

fish, the dominant microbiota (*Shewanella spp., Photobacterium spp., Pseudomonas spp., Acinetobacter spp*) turned to *Photobacterium spp.* Aerobic bacteria counts (ABC) of fishery products have become less valuable spoilage indicators. Spoilage activity was found to be 6-200 times higher in *Photobacterium spp.* than in *Shewanella spp*. and *Pseudomonas spp*. when measured as the yield factor for total volatile basic nitrogen (TVB-N) formation. Furthermore, *Photobacterium carnosum* was discovered to be the primary spoilage microrganism that limited the shelf-life of iced products, whether stored in air or MAP (Sorensen et al., 2020). Some enteric pathogenic bacteria species such as *Clostridium spp., Vibrio spp., Salmonella spp., Escherichia spp., Shigella spp.* can all be found in polluted marine environments. Live fish and shellfish can also contain these pathogens. Furthermore, the microorganisms found in seafood can cause a variety of illnesses and even death (Kılınç and Besler, 2014). Pathogens are classified into different types based on their ability to survive heat treatment and grow at cold temperatures. Vegetative bacteria cannot grow at cold storage temperatures. Pathogens such as *S. aureus, Vibrio spp., Campylobacter spp., Salmonella spp.* and *E. coli,* are killed by pasteurisation, and growth is seriously limited in agreeably refrigerated foods. *Aeromonas, Listeria,* and *Yersinia* species are examples of vegetative bacteria that are rendered inactive by pasteurising heat treatments but whose cells survive in low heat treatments and can multiply during cold storage. Psychrotrophic spore-forming bacteria (psychrotrophic *Bacillus cereus* and non-proteolytic *C. botulinum*) that can grow at low temperatures and survive pasteurisation. Mesophilic spore-forming bacteria (*C. perfringens,* mesophilic *B. cereus,* and proteolytic *C. botulinum*) that can withstand cooking but cannot multiply at low temperatures (Ünlütürk and Turantaş, 2003; Stringer and Metris, 2018; Kılınç, 2020).

Pathogens (*Listeria monocytogenes, Escherichia coli, Vibrio spp., Salmonella spp.,* etc.) can survive in food products that have been lightly processed, such as sous-vide cooked, smoked or gravad. The raw material quality is critical in these products. For this reason, these products should be consumed as soon as possible after preparation. Products not for directly consumption require a pasteurising heat treatment followed by cold storage for preservation and safety (Kılınç, 2001; Meljholm, 2007; Stringer and

Metris, 2018). Ready-to-eat foods that have not been heat treated before consumption are susceptible to foodborne hazards and spoilage, so a method for monitoring safety would be benefical (Ivorra et al., 2016; Kafa and Kılınç, 2022).

4. THE PREDICTIVE MODELS USED IN BACTERIAL GROWTH OF FISHERY PRODUCTS DURING STORAGE

Mathematical predictive models are often used to estimate microbial growth of different bacteria species in various types of food products (Teleken et al., 2018). Rosa et al (2000) investigated predictive models for assessing the risks to human health of *L. monocytogenes* in fishery products. One study's goal was to use predictive modeling for estimating the microbial growth as an important key component in evaluating the numeric bacteriological contamination of ice-stored sea bass (*Dicentrarchus labrax*). In this study, different mathematical techniques were examined to determine variations in bacterial growth in the whole and ungutted sea bass stored on ice. The authors confirmed the presence of specific spoilage bacteria (SSB) in the gills. Furthermore, by better understanding the microorganisms that cause the deterioration and spoilage of sea bass, the use of these predictive models reported to be improved food safety control (Iruzubieta et al., 2014). Predictive modeling techniques for *Aeromonas hydrophila* on fresh tuna (*Thunnus orientalis*) as a role of varying storage conditions (2-15 °C) were also developed by Kim et al. (2022). In this study, the main models were found fit well (R2; 0.97-0.98) with the Baranyi model at these storage temperatures to get specific growth rate (SGR) and lag time (LT). The authors reported that these predictive models not only could be used to predict *A. hydrophila* development on fresh tuna at different cold storage temperatures but also, for preserving safe levels of *A. hydrophila* throughout raw fish (*Thunnus orientalis*) of a processing and distribution (Kim et al., 2022). A modified Richards model was developed to estimate non-isothermal and thermal growth of *Pseudomonas spp*. The model's fit results demonstrated that it could accurately describe and estimate *Pseudomonas* growth curves under varying temperatures regimes. However, the effect of the shape parameter on the growth curve requires further investigation reported by Teleken et al., (2018). Another study's goals were to evaluate the effect of osmotic

concentration of solute on heat and mass transfer kinetic models during drying and to develop a statistical model for the effect of water activity (aw) and storage temperature on the shelf-life of osmotically drying gilthead seabream (*Sparus aurata*) fillets. To describe aw as a component of maltodextrin concentration in the osmotic pressure difference and preparation time, a first-degree polynomial model was used. This mathematical model was created using a modified Arrhenius-type equation to assess the mixed impact of temperature and water activity on increase rate of *Pseudomonas spp.* in fish throughout chilled storage (Tsironi and Taoukis, 2014). The mathematical model was also created to investigate consumer listeriosis risk. *L. monocytogenes* prevalence was calculated by predicting bacterial increases in foods at different temperatures. The model is based on a two-state Markov chain that describes multiple days of consumption. The risk of population was then estimated using a Poisson distribution, which took into account both the regular possibility of buying a contaminated product and the total cumulative potential of infectious disease from its use. The authors concluded from this research that food storage and handling processes were very critical in protection from listeriosis. Therefore, the estimation model used in this study allowed for the evaluation of the combined effects of these effects (Pasonen et al., 2019). In another study, the observed growth curves were used to assume the maximum increase rate of *L. monocytogenes* using the Baranyi and Roberts models. The Ratkowsky square root model was also used to determine the effect of storage temperature on max growth rate of *L. monocytogenes*. The improved models were approved using *L. monocytogenes* growth data from sea bass and sea bream fillets stored at different temperatures. The current study presented reliable and valid predictive models for *L. monocytogenes* development in Mediterranean fish species, which were indicated to be implemented in shelf-life and microbial risk management studies (Bolivar et al., 2018).

5. THE PREDICTIVE MODELS USED IN THE FRESHNESS, QUALITY, AND SHELF-LIFE OF FISHERY PRODUCTS

In order to examine the quality and freshness of fishery products in a shorter period of time, technology is constantly evolving. Time Temperaure Integrators (TTI) were used as smart labels in one report, which cost-effectively and individually gave rise to monitoring the history of food product temperature reflecting their quality during the cold chain of frozen seafood. (Giannoglou et al., 2014). The shelf-life visibility and Temperature monitoring, as well as the Least Shelf-life First Out (LSFO) stock strategy, were reported to be very critical components of perishable food shelf-life estimation and chill/cold chain logistics for both workers and cold chain managers to maintain quality of foods for longer and reduce economic losses in another report by Qi et al. (2014).

Using advanced technologies to determine the freshness and quality of fish/fishery products can lead to the development of precise models for estimating the quality and shelf-life of various fish products under various conditions. Thus, these predictive models developed under different conditions for each fishery product can provide an accurate estimate of the quality and shelf-life of fishery products without requiring any analysis. Numerous studies have been conducted on the predictive models used in the estimation of freshness, quality, and shelf-life of fishery products. Some of these studies are summarized below.

Using the Excitation-Emission Matrix characteristic components (EEM), back-propagation neural networks (BPNNs) and radial basis function neural networks (RBFNNs) models were improved for measuring the freshness of bighead carp head (*Hypophthalmichthys nobilis*) storage at varying temperatures. The authors confirmed that PARAFAC's EEM-BPNNs model of bighead carp eye fluid has a high capacity for measuring fish freshness at different storage tempeartures (Shi et al., 2022). In another study, the authors showed that combining the fluorescence Excitation-emission matrices (EEM) of the surface-containing scales and eyeball provided the best observing of Japanese dace fish (*Tribolodon hakonensis*) freshness according to the results of partial least square regression (PLSR) (Omwange et al., 2022).

In one study, at isothermal temperatures ranging from 0 to 15°C, the microbiological flora of air-packed sea bass (*Dicentrachus labrax*) was examined and modelled using the four-parameter Logistic equation. Shelf- life correlations, kinetic models, and N0 data were integrated into the shelf-life control system (SLDS), which was demonstrated to be an appropriate tool for controlling the cold chain of marine cultivated sea bass, resulting in better fish quality for the consumers (Koutsoumanis et al., 2002). Another research adopted sensory data and detection time into a predictive regression model that predicted the leftover shelf-life of chilled fishery products. Because bacterial activity in packaged fish varies more, this study revealed that this predictive linear model could predict the shelf-life of iced whole cod but not for vacuum-packed fillets (Jorgensen et al., 1988). An empirical model was developed in the other study to estimate the shelf-life of tropical shrimp (*Penaeus notialis*) in varying storage temperatures. The research improved our understanding about tropical shrimp spoilage and laid the groundwork for the improvement of methods and tools to develop the quality management of shrimp, as reported by Dabade et al (2015). The shelf-life and freshness estimation modeling of European sea bass (*Dicentrarchus labrax*) based on electronic nose and chemical methods were also investigated by (Limbo et.al.2009). A collaborative effort (consumer advertising and sales point management), according to this study, could reduce exposure temperature by 1-2 degrees Celsius and justify extending shelf-life to 2-3 days after buying (Limbo et al., 2009).

The other studies conducted on the predictive models to assume the shelf-life of fishery products during storage as follows. Giarratana et al. (2022) reported that the development of mathematical model based on changing storage temperature conditions of the Atlantic mackerel (*Scomber scombrus*) were obtained to estimate the shelf-life, whereas sardine (*Sardina pilchardus*) quality was assessed by Bulat et al (2020) using regression models. The Pearson correlation r between storage times for meat, gill, and intestine quality and shelf-life for sardines stored at 4 °C (model 1) was reported to be 0.86, and r=1 for storage times for meat and gill quality at 4 and 10 °C, respectively (model 2). It is recommended in this study that two models were very suitable for estimating the quality and shelf-life of sardines (Bulat et al., 2020). The natural microflora of different spoilage

microorganisms of Mediterranean gilt-head seabream (*Sparus aurata*) was studied in one study under different storage conditions ranging from 0 to 15°C. In this study, Koutsoumanis (2001) used a Belehradek type model to assess the effects of temperature on the growth rate of pseudomonad species. The average difference (%) between shelf-life estimated based on the growth of *Pseudomonas* species and shelf-life experimentally determined using sensory analysis for all temperatures tested was 5.8%, demonstrating that this model can be used to exactly estimate Mediterranean gilt-head seabream quality in real-world conditions (Koutsoumanis, 2001). In another study, Koutsoumanis and Nychas (2000) used a systematic experimental procedure to develop a model for assuming the quality and shelf-life of gilt-head sea bream (*Sparus aurata*) in the chill chain. A conductance assay was also applied to shorten the time needed for the counting the initial number of pseudomonads, which was very critical information for shelf-life estimation. This method produced results in one-fourth the time, when compared with traditional microbiological tests (Koutsoumanis and Nychas, 2000). Shelf-life was predicted using the increasing rate of specific spoilage organisms (SSO) in model substrates. The influence of carbon dioxide (CO2) on the growth rates of *Shewanella putrefaciens* and *Photobacterium phosphoreum* was also modeled. Validation of kinetic models was preferred for comparing bacterial growth rates determined in product and model system experiments by comparing shelf-life estimations with shelf-life determined in product experiments by sensory evaluations. Kinetic modeling was discovered to be extremely useful for both evaluation and prediction in this study. It was proposed to iteratively develop kinetic shelf-life models and predict microbial fish spoilage (Dalgaard, 1995). In order to develop models for assessing microbial quality and determining silver pomfret (*Pampus argenteus*) shelf-life, the impediometric technique was used as fast sensitive method to determine the relationship between impedance detection times (IDTs) and standard reference mesophilic and psychrophilic plate counts of fish. The calibration curves and equations for the two methods were generated using linear regression models. IDTs had a strong relationship with psychrophilic (r=-0.9614) and mesophilic (r=-0.9547) bacterial loads. The impediometric technique, according to the findings of this study, can be used to properly assess the bacterial loads of silver pomfret and define its shelf-life as seafood

(Fazlara et al., 2014). To investigate the effect of varying temperatures on spoilage of *Litopenaeus vannamei*, fresh shrimp were taken from a tropical farm and stored at different storage conditions (1 °C ice, 4 °C refrigerated, and 30±2 °C room temperature). The samples were tested for sensory, chemical, and microbial properties at regular intervals. The shelf lives of the samples in ice storage, refrigerated storage, and at room temperature were estimated to be 16 days, 12 days, and 10 hours, respectively. The fitness of three Relative Rate of Spoilage (RRS) models was tested to define the shelf-life of *Litopenaeus vannamei*. The Arrhenius model was discovered to be more appropriate in this study (Don et al., 2018). Taoukis et al (1999) developed a systematic approach for fish shelf-life modeling and time temperatures interactions (TTI) selection in order to plan and implement an effective quality observing scheme for the fish chill chain. The temperature behavior of the Mediterranean fish (*Boops boops*) boque's natural microflora was studied. Additionally, the growth of spoilage bacteria *Shewanella putrefaciens* and *Pseudomonas spp.* was modeled and associated with the sensorial shelf-life. The temperature dependence of maximum growth rates was modeled using Arrhenius and square root functions. Using individual varying temperature tests, microbial activity and shelf-life models were confirmed in active storage conditions. (Taoukis et al.,1999).

Table 4. Some of the Predictive Models Used in Fishery Products

Fish Species	Model Type	Prediction	Publication
Mediterranean fish boque *(Boops boops*)	Arrhenius and square root functions	Determination growth of the spoilage bacteria *Shewanella putrefaciens* and *Pseudomonas spp.* was modeled and correlated with sensorial shelf-life.	(Taoukis et al.,1999).
Mediterannean gilt-head seabream (*Sparus aurata*)	Belehradek type model	Shelf-life predicted based on the microbiological growth of *Pseudomonad* species.	(Koutsoumanis, 2001).
Silver pomfret (*Pampus argenteus*)	Linear regression models	Prediction of the microbial quality and determination of the shelf-life	(Fazlara et al., 2014).
Fresh shrimp (*Litopenaeus vannamei*)	Arrhenius model	Determination of the shelf-life and the influence of the temperature on spoilage of fish	(Don et al., 2018).
Sardine (*Sardina pilchardus*)	Regression models	Determination the quality and shelf-life of meat, intestine and gill at 4 and 10^{o}C	(Bulat et al., 2020)
Bighead carp head (*Hypophthalmichthys nobilis*)	Neural networks models	Prediction of fish freshness under variable storage conditions	(Shi et al., 2022).

6. THE PREDICTIVE MODELS USED IN THE BACTERIAL GROWTH AND SHELF-LIFE OF PROCESSED FISHERY PRODUCTS DURING STORAGE

The following predictive models were used in the studies to estimate the bacterial growth and shelf-life of processed fishery products during storage. Kılınç et al. (2021) developed regression and grey models to estimate the development of total viable bacteria count on frozen squid rings during the thawing process. In this study, the regression model was reported to be the best model for estimating the microbiological growth and the shelf-life of frozen squid rings during thawing at different times and temperatures (R2adj = 0.918). The grey model was also mentioned to be a very good alternative when there was limited data and time-dependent data were measured. The both suggested modeling techniques were found to be very effective for predicting bacterial growth (Kılınç et al., 2021). In their study, Giannoglou et al. (2019) reported that numerical techniques that define the impact of temperature, enzyme concentration, and duration of storage on temperature interactions TTI response were approved under different conditions. The necessary TTI design to accurately observe the shelf-life of the various smoked products was examined by using advanced predictive models. At 5 °C, vacuum-packed smoked salmon, trout, and eel had shelf lives of 2, 11, and 7.5 weeks, respectively (Giannoglou et al., 2019). A k-nearest-neighbors (KKN) model was improved to classify salmon tissue. The use of partial least squares models confirmed the importance of spatial segmentation prior to developing a shelf-life model. These findings indicated that the developed method holded great promise as a non-destructive method for determining the shelf-life of vacuum-packed cold stored smoked salmon fillets (Ivarro et al., 2016). In one study, rainbow trout (*Onchorhyncus mykiss*) quality changes were evaluated by Yin et al. (2022) at different storage temperatures in modified atmosphere packaging (MAP), vacuum packaging (VP) and common packaging (CP). In this study, except for the TBA value in VP, the zero-order model or empirical exponential model fit the experimental data well. The predictive models used to estimate the shelf-life of rainbow trout in various packaging methods at temperatures ranging from 4 to 15 degrees Celsius were obtained (Yin et al., 2022). In another study, freeze-dried (FD) and hot-air-dried (HD) *Penaeus vannamei* shelf-lives were predicted using

the Arrhenius Equation. The shelf life of FD shrimp was expected to be 1.47 times that of HD shrimp. Throughout storage, FD shrimp had lower levels of oxidation parameters such as thiobarbituric acid-reactive substances value (TBARS) and peroxide value (POV) than HD shrimp. The authors confirmed that FD shrimps had higher quality during storage and a longer shelf-life than HD shrimps as a result of this study (Li et al., 2019).The effect of gamma irradiation on psychrotrophic microorganisms in vacuum-packed squid (*Illex argentinus*) rings stored at 4-5 °C was investigated by (Tomac et al., 2013). In this regard, a polynomial expression and modified Gompertz model based on storage time and irradiation dose were used. Both proposed models accurately predicted psychrotrophic bacteria behavior as influenced by gamma irradiation, allowing exact shelf-life predictions for doses up to 5.3 kGy (Tomac et al., 2013). Lorentzen et al. (2014) investigated the shelf-life of cooked red king crab (*Paralithodes camtschaticus*) clusters (shoulder plus three legs) during chilled storage. The authors of this study investigated the chemical, sensory, and microbiological changes in clusters during storage in order to detect spoilage and predict shelf-life. In five days stored leg meat, the maximum levels of TVC and *Pseudomonas spp*. were 4.6 and 3.7 log CFU/g, respectively. The odor of the shoulder joint was used to estimate the flavor of crab leg meat using exponential regression analyses (Lorentzen et al., 2014). Another study determined the Gompertz model parameters and their temperature dependence as a quadratic equation for applications in fish cake shelf-life prediction. At 10, 4 and 0^0C, the predicted shelf-life values for fish cake used in this study were 3.8, 5.5, 6.9 days, respectively. Based on statistical analyses that revealed accuracy and bias factors, the shelf-life prediction equation was determined to be appropriate. The authors came to the conclusion that the Gompertz predictive model can be used to assume the shelf-life of fish cake (Kang and Song, 2013).

Table 5. Some of the Predictive Models Used in Processed Fishery Products

Fishery Product	Model Type	Prediction	Publication
Fish cake	Gompertz model	Based on statistical analyses that revealed accuracy and bias factors, the shelf-life prediction	(Kang and Song, 2013).
Processed Squid *(Illex argentinus*) rings	Gompertz model	*Psychrotrophic* bacteria behavior was modeled when gamma irradiation was used to improve the shelf-life	(Tomac et al., 2013).
Cooked red king crab *(Paralithodes camtschaticus)*	Exponential regression analyses model	Besides sensory and microbiological changes to observe spoilage during storage conditions, using exponential regression analyses for predicting shelf-life	(Lorentzen et al., 2014)
Processed shrimp (*Panaeus vannamei*)	Arrhenius model	Prediction of the shelf-lives of freeze-dried and hot-air-dried shrimp	(Li et al., 2019)
Frozen squid (*Illex argentinus*) rings	Regression models	Prediction of the microbiological growth on frozen squid rings during the thawing process at various temperatures and times	(Kılınç et al., 2021)
Packaged rainbow trout *(Oncorhynchus mykiss)*	Zero-order model, empirical exponential model	Prediction of shelf-life of fish in various packaging methods at temperatures ranging from 4 to 15ºC	(Yin et al., 2022)

7. CONCLUSION

Microbiological tests are both costly and time consuming. However, the predictive models used to estimate the microbiological growth and shelf-life of fishery products are time and cost effective. The rise in such studies is significant in terms of assessing fish quality and shelf-life.

In parallel with the increasing population and nutritional needs, researches in the field of food microbiology are increasing in importance in order to provide food products safety.

In the food industry, the approach of mathematical models has become necessary to better understand microbial behavior and investigate the accuracy of applied processes based on the actual shelf-life and microbial safety of foods. Today, depending on the developing computer technology, the possibilities of using predictive models as software in food microbiology have also increased.

However, the use of existing modeling software in the food industry is still not common. Through this section, users were informed about predictive models, their use in terms of bacteral growth and shelf-life of fishery products during storage, and the criteria to be considered in choosing a good model.

As a result, in the future, estimating the shelf-life of food and aquatic products using predictive models rather than microbiological analyses and estimating bacterial species that may pose a risk in products will be preferable methods to those used today. Predictive models are expected to be a very useful and valuable tool in both research and industrial food conservation processes in the future. It is believed that the development of many software that provides faster and more reliable information from predictive models will continue.

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CHAPTER 5

BIOPLASTIC RAW MATERIALS OBTAINED FROM AQUATIC ENVIRONMENTS

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1. INTRODUCTION

It's like the whole world is wrapped in plastic. Plastics are among the indispensables of our daily lives and are used in household appliances, cars, sports equipment, office and information technologies and packaging. The decomposition time of petroleum-derived plastics in nature is very long. For this reason, they create a waste problem and are difficult to separate in recycling. There are several solutions to the environmental pollution problem caused by non-degradable plastic waste. These are incineration, recycling or producing and using biodegradable plastics.

With the combustion removal method, harmful gases (such as hydrochloric acid and hydrogen cyanide) are released into the air we breathe. This situation brings with it factors that threaten human health. Emission of these gases also poses a major threat to the global warming problem by causing the greenhouse effect. It takes a long time to sort plastic waste materials in recycling applications. It is also an expensive application. For all these reasons, bioplastic production, which is an alternative application, appears as a solution proposal.

Native polymers like proteins, polysaccharides and lipids or a mixture of the ingredients play a role in the readying of biocomposites. (Figure 1). Biocomposites formed from plastics of biological origin called 'biopolymer' or 'bioplastic' are called 'green composites'.

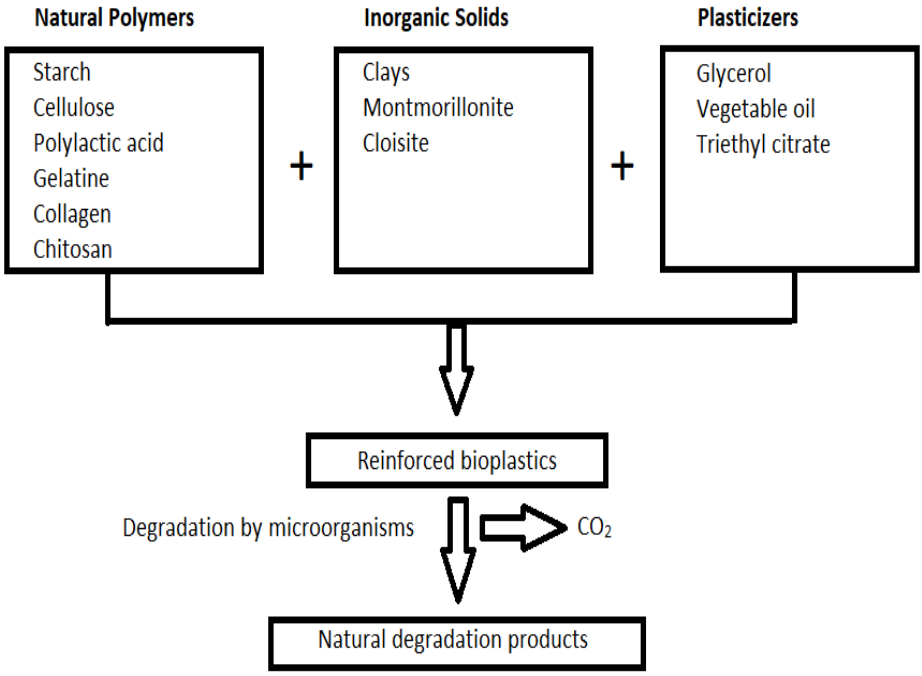

Figure 1. Additives that can be used during the formation of bionanocomposites (Dursun et al., 2010).

Bioplastics can be classified into three categories (Figure 2). Except the fossil based non-biodegradable plastic, rest three are considered under the category of bioplastics. True bioplastics (bio-based and biodegradable) are a very valuable material in the plastics industry. Because;

- It is completely degradable in nature.
- Eliminates the need for petroleum.
- It is created utilizing renewable energy sources.
- It has the physicochemical characteristics of synthetic plastics.

Of all the biodegradable plastics, only poly(3-hydroxyalkanoate) is completely degraded by microorganisms when it accumulates inside the cell during uneven growth conditions.

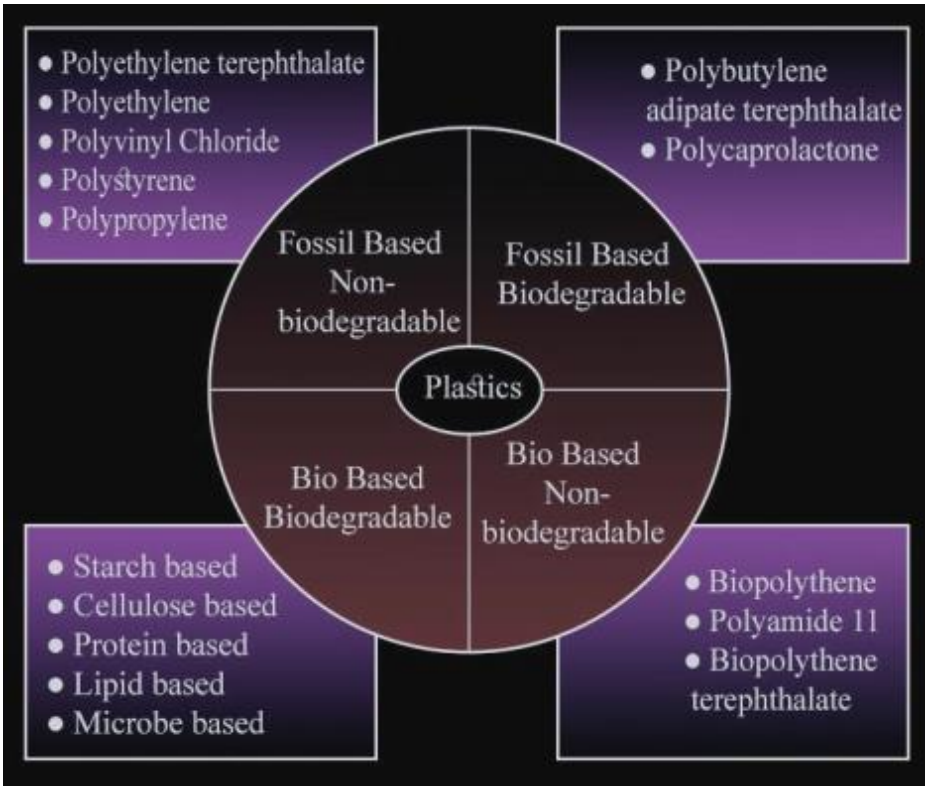

Figure 2. Types of Plastics, Their Degradability and Examples (George et al., 2021).

2. AQUATIC RAW MATERIALS

2.1. Raw Materials From Aquatic Microorganisms

Bioplastics can be made from polysaccharides produced by algae. Phytoplankters secrete mucus-like organic substances under certain conditions or when they die, causing mucilage formation. In 2021, mucilage was seen in the Sea of Marmara and caused serious problems in many respects. Mucilage has the potential to be converted into a plastic material, thanks to its polysaccharide structure. Bioplastics to be produced from mucilage have been presented as a disposal method for the removal of mucilage. When polyvinyl alcohol, a biodegradable polymer, was compared with mucilage, it was observed that mucilage was better in terms of mechanical properties, UV protection and antibacterial activities.

Biodegradable plastic films were prepared from Spirulina, a cyanobacterium. Spirulina has been used in Spirulina/PVA mixtures in the

presence or absence of microbial transglutaminase crosslinker at various concentrations. In addition, Spirulina was used as a single component at various plasticizer and enzyme concentrations. In addition, films modified or unmodified with microbial Tranglutaminase were prepared by extracting the proteins of Spirulina.

2.2. Raw Materials From Shellfishes

Millions of tons of crustaceans such as shrimp, mussels, oysters, lobster and crab are produced worldwide each year. The edible parts of them are very rich in protein. However, other inedible parts (waste) constitute approximately half of the body weight. Shells are an important source of polysaccharides (chitin) and protein. Chitin is a linear homo-polysaccharide composed of b-(1,4)-linked N-acetyl-D-glucosamine units. Chitosan is not extensively found in environment and is a deacetylated configuration of chitin get from crustaceans fall-out. Chitosan possesses characteristic properties such as solvability in weak acids, film making capability, pH sensitiveness, biological degradability and biological compatibility, non-antigenicity, non-toxicity and being economical. It is therefore gaining attention to uses at the aliment, biomedical and dye industries. In the context of the food industries, chitosan-based composite films and coatings are produced to extend the post-harvest life of fruits and vegetables.

Chitosan is used in the preparation of biodegradable films, blends, coatings, composites and nanocomposites. For this reason, it attracts the scientific world and different sectors.

Crustacean shells generally consist of 20-30% chitin, 30-40% protein, 30-50% calcium carbonate/phosphate. Apart from this, it also comprises several pigments (astaxanthin, cantaxanthin, lutein or β-carotene).

2.3. Raw Materials From Fishes

Industrial fish processing wastes can constitute up to 70% of the initial weight of the fish. This waste is noted a high-quality and inexpensive raw material. It often cause environmental, health and economic damage. Fish waste is converted to bioplastic for use. The wastes included in the production contribute to the reduction of environmental pollution.

There are opportune characteristics, such as plasticity and elasticity, and mechanical and airtight barriers, in bioplastic production from fish proteins (Figure 4).

Chitosan has been obtained from fish scales and other wastes of fish, the squid gladius (*Loligo vulgaris*). Myofibrillar proteins, collagen and gelatin were obtained from the fish by-product. Biopolymers extracted from fish by-products have been effective in obtaining bioplastics. Thus, the environmental impact of waste disposal can be reduced.

In addition to chitin, the existence of collagen in the construct of fish scales was also confirmed. Among natural amino polysaccharides, chitin is the most plenty, following cellulose. Chitin extracted from the scales of carp (*Cyprinus carpio* L.) has been characterized by its functional properties.

The stages of bioplastic production from fish scale are as follows. First, the fish will be collected and scaled. Then the melted fish flakes are leavened in a glass utensil at a room temperature of 26-38°C for three months. As leavening happens, bacteria augmented on the flakes. After the leavening procedure, the leavened flakes are subjected to cell disruption, washing, centrifugation and drying to purify the biopolymer. Then, the purification of the biopolymer takes place with the steps of cell dispersion, washing and centrifugation, drying. The final step is bioplastic production (Figure 3).

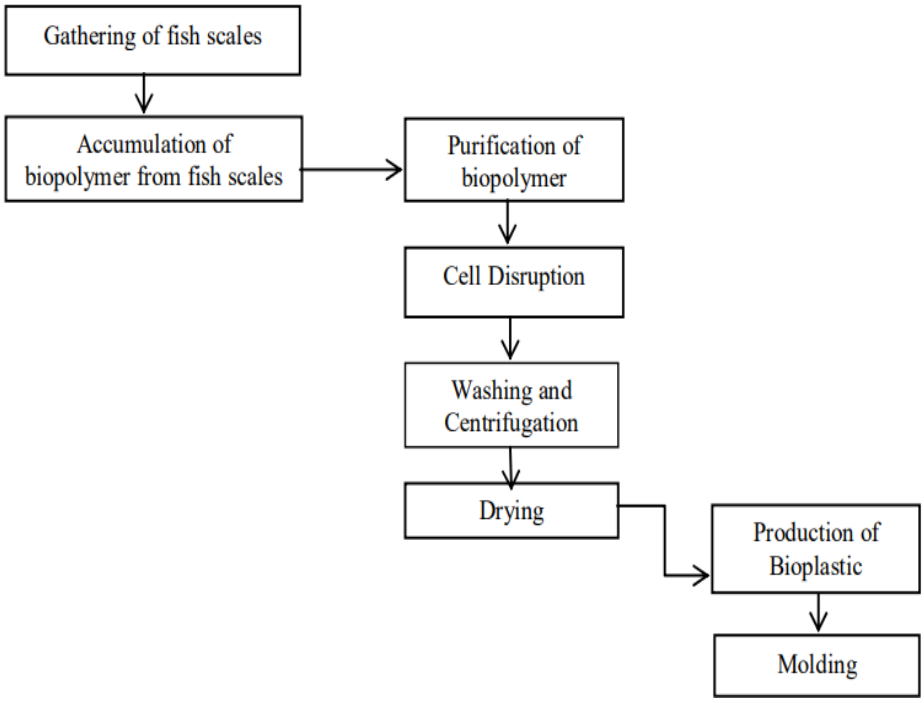

Figure 3. Schema of process of bioplastic (Varna et al., 2019).

Bioplastics containing 0.79% myofibrillar proteins (*Brachyplatystoma rousseauxii* wastes) and 40% plasticizer were found to be homogeneous, transparent, strong, flexible. Permeability of water vapour and solvability of the bioplastics were low.

Bioplastics were obtained by adding different amounts of chitosan (2%, 3%, 4%) and fish bone gelatin (5%, 10%, 15%, 20%). The most efficient mixture in terms of thickness (0.072 mm), transparency (2.09), tensile strength (19.05 MPa), percent elongation (28.333%) and biodegradability is the package produced with the addition of 2% chitosan and 5% gelatin concentration. Bioplastics made from this mixture degraded 99.84% after 14 days.

In a study investigating the effect of microcrystalline cellulose on bioplastic, fish scale powder, wheat gluten, silica, and glycerol were used in the mixture. Microcrystalline cellulose improved the chemical stability and tensile strength of the bioplastic. As a result of the biodegradability tests, the weight of the bioplastic decreased by 35% in 14 days.

Figure 4. Optimized bioplastic prepared using myofibrillar proteins from gilded catfish (Araújo et al., 2018).

Biodegradable films have been developed from a blend of fish gelatin and myofibrillar. For this, the fish proteins were removed from the fillet leftover of *Macrodon ancylodon*. According to mechanical analysis, myofibrillar protein films are more resistant to traction, while gelatin is more flexible. Water vapor permeability and solubility were found to be low in the blend biofilms. These films are transparent, mechanically strong and flexible. This confirmed that the properties were improved by mixing the polymers. The analyzes revealed the coaction of gelatin and myofibrillar protein chains with each other. In this way, it was revealed that the films formed a cohesive and strengthened matrix resulting in great thermal resistance. It has been determined that bioplastic food packaging can be produced from the mixture of polymers.

3. CONCLISION

Many studies have been conducted on the production of bioplastics with raw materials obtained from aquatic environments. Collagen, gelatin, chitin and chitosan in aquatic microorganisms, fish and crustaceans play an important role in the production of bioplastics.

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CHAPTER 6

DETERMINATION OF THE EFFECTS OF COOKING AND MARINATING PROCESSES ON THE FATTY ACID AND AMINO ACID PROFILES OF SQUID RINGS

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1. INTRODUCTION

Marine fishery products are very important sources not only because of proteins, fats, fat-soluble vitamins, but also they have a good source of n-3 polyunsaturated fatty acids (PUFAs). The proportion of the monounsaturated fatty acids (MUFAs), total saturated fatty acids (SFAs), and polyunsaturated fatty acids (PUFAs) change according to the different fish-feeding habits (Wang et al., 2020). Additionally, the proportion of fatty acids changes according to the different organs of the same species of the fishery products. The fatty acid profiles in muscle and gonads of the brown crab (*Cancer pagurus*) were dominated by PUFA, while hepatopancreas of this species was richer in MUFA and SFA (Barrento et al., 2010). Additionally, processing also affects the ratio of fatty acid composition of fishery products. The proportion of MUFAs did not changed significantly in *Pangasius hypophthalmus* fish after smoking, but the proportions of PUFAs and SFAs increased significantly in smoked fillet compared to fresh filet. The ω-6 and ω-3 fatty acid ratios and the ω-6/ω-3 ratio of fresh *Pangasius hypophthalmus* fillets were 33.23, 4.79, and 6.94, respectively; while the values in smoked fillets were 38.40, 5.45 and 7.05, respectively (Sokamte et al., 2020). Fishery products are rich in biologically important long-chain PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Farvin and Surendraraj, 2019).

Fish fatty acids the fish European eel (*Anguilla anguilla*) during the growth of the contents more eicosatrienoic acid, linolenic acid, tricosanoic acid, and arachidonic acid, whereas the lysine, threonine, alanine, serine, proline, and arginine contents were highest in smaller fish (Gomez-Limia et al., 2021). The total fatty acid of raw Papuan cork fish *Channa striata* meat was 67.28%, whereas the total amino acid of raw fish meat was 67.49% (Pasaribu et al., 2020). The fatty acids of *Prochilodus scrofa* were palmitic, stearic palmitoleic, linoleic, linolenic acids, and oleic whereas the amino acid composition of *P. scrofa* proteins was high in methionine and lysine, but low in cysteine/cystine (Maia et al., 1983). The most abundant essential amino acids of chub mackerel (*Scomber colias*) changed according to seasonality, lysine and leucine contents ranged from 1270 mg/100 g in August to 1820 mg/100 g in November. The other essential amino acids' contents changed from 500 to 1200 mg/100 g (Motta et al., 2021).

Escolar fish (*Lepidocybium flavobrunneum*) contained the highest amount of the essential amino acids (70.63±3.61 mg/kg), while oleic and elaidic fatty acids of this fish were the most abundantly present (28.115±4.44%) (Dordevic et al., 2016). The major amino acids of *Channa spp*. were lysine, glutamic acid, aspartic acid, and ranging from 9.7% to 21.7%, and the most abundant fatty acid in *Channa* spp. was C16:0. C22:6, C18:1, and C18:0 were also determined that ranging from 25.6% to 30.4% (Zuraini et al., 2006). The highest content of SFA of paper squid (*Loligo edulis*) was palmitic acid, which was 7.45%, whereas the highest content of MUFA of squid was oleic acid, which was 0.79%. In addition to this, the highest content of PUFA of squid was docosahexaenoic acid, which was 22.57% (Apituley et al., 2020). SFAs content of European squid (*Loligo vulgaris*) was increased but MUFAs and PUFAs contents were decreased during frozen storage of squid (Atayeter and Ercoskun, 2011). Docosahexaenoic, palmitic, and eicosipentaenoic acid were the most abundant fatty acids in the Mediterranean (cuttlefish, octopus, and squid). The amount of n-3 FAs was higher than SFA, MUFA, and n-6 FAs. Despite the fact that cephalopods contain a small amounts of fat, they were found to be quite rich in n-3 fatty acids (Zlatanos et al., 2006).

n-3 PUFAs are very essential fatty acids that have a very important role in growth and development throughout the life cycle of humans. Therefore, in terms of the presence of all essential amino acids, high concentrations of DHA and EPA, as well as the high n-3/n-6 ratio of fishery products close to the recommended value, they should be included in the diet of all people. It characterize this product as important for human food, and also for human nutrition the objective of the prevention of cardiovascular diseases (Dvoretsky et al., 2021). The fatty acid composition of fish shown a high content of PUFA, which can be obtained from 0.27 to 1.79 g for the sum of EPA and DHA (Douny et al., 2021). The amino acid can also be taken into the body from the different ways of the processed or cooked fishery products. Fatty fish have 81 % essential amino acids that were the recommended intakes of the individual portions (Motta et al., 2020). Aquatic meats represent very important sources of dietary protein and micronutrients. Accordingly, it is important to understand how these products are processed in order to prevent the loss of nutritional quality (Luo et al., 2018).

Marinating is a food-preserving technique that fishery products that can be preserved with vinegar and spices, which gives rise to a decrease in the pH and thereby retards the growth of bacteria (Toyohara et al., 1999). There have been made many studies about the determination of the fatty acid and amino acid compositions of the different marine fishery products (Maia et al., 1983; Zlatanos et al., 2006; Zuraini et al., 2006; Barrento et al., 2010; Apituley et al., 2020; Wang et al., 2020; Dvoretsky et al., 2021; Gomez-Limia et al., 2021; Loppies et al., 2021; Motta et al., 2021). Additionally, limited studies have been conducted about the definition of the processing effects on the fatty acid and amino acid contents of the fishery products (Ozden, 2005; Türkkan et al., 2008; Anggo et al 2015; Pasaribu et al., 2020; Sokamte et al., 2020). However, no literature has been found on the evaluation of the effects of cooking and marination processes on the amino acid and fatty acid profiles of squid rings. In addition, the linear and quadratic effects of the different dietary protein levels on some fatty acids have been investigated in the literature (Xu et al., 2018). From a similar point of view, it was possible to model the ratio of amino acids and fatty acids depending on time.

Therefore, the aim of this study was to determine the effects of cooking and marination processes on the fatty acid and amino acid profiles of the squid rings. Another purpose of the study was to investigate the most suitable statistical model for some of the important amino acid and fatty acid profiles of squid rings with appropriate regression analysis.

2. MATERIAL & METHODS

2.1. Material

Frozen squid rings (*Loligo vulgaris*) were obtained from the seafood processing factory of İzmir province of Turkey. After arriving at the Ege University Fisheries Faculty, they were divided into different groups into the laboratory of Fish Processing Technology. Group A consisted of the marinated and un-marinated squid rings, whereas Group B included cooked squid rings as well as cooked marinated squid rings. Frozen squid rings were cooked in the water bath, which was adjusted at 50°C for 5 minutes containing hot water. The marination process was also applied to the cooked squid rings.

The formulation of the groups are given in Table 1-2. The groups of un-marinated and marinated squid rings stored at 4°C are shown in Table 1, wheras

the groups of cooked and cooked marinated squid rings stored at 4°C are seen in Table 2.

Table 1. The groups of marinated and un-marinated squid rings in different times stored at 4°C

A Groups					
Marinated hour	**un-marinated frozen squid rings**	**Marinated frozen squid rings**			
		10+90 (lemon juice+mineral water) ml /100g squid rings	**90+10 (lemon juice+mineral water) ml /100g squid rings**	**50+ 50 (lemon juice+mineral water) ml /100g squid rings**	**100+100 (lemon juice+mineral water) ml/100g squid rings**
1 hour	Group 2A	Group 9A	Group 16A	Group 23A	Group 30A
3 hour	Group 3A	Group 10A	Group 17A	Group 24A	Group 31A
6hour	Group 4A	Group 11A	Group 18A	Group 25A	Group 32A
12 hour	Group 5A	Group 12A	Group 19A	Group 26A	Group 33A
24 hour	Group 6A	Group 13A	Group 20A	Group 27A	Group 34A
48 hour	Group 7A	Group 14A	Group 21A	Group 28A	Group 35A
72 hour	Group 8A	Group 15A	Group 22A	Group 29A	Group 36A
Group 1: Frozen squid rings, Group A: Un-marinated and marinated frozen squid rings					

Table 2. The groups of cooked and cooked marinated squid rings in different times stored at 4°C

B Groups					
Marinated hour	**Cooked frozen squid rings**	**Cooked marinated frozen squid rings**			
		10+90 (lemon juice+mineral water) ml /100g squid rings	**90+10 (lemon juice+mineral water) ml /100g squid rings**	**50+ 50 (lemon juice+mineral water) ml /100g squid rings**	**100+100 (lemon juice+mineral water) ml/100g squid rings**
1 hour	Group 2B	Group 9B	Group 16B	Group 23B	Group 30B
3 hour	Group 3B	Group 10B	Group 17B	Group 24B	Group 31B
6hour	Group 4B	Group 11B	Group 18B	Group 25B	Group 32B
12 hour	Group 5B	Group 12B	Group 19B	Group 26B	Group 33B
24 hour	Group 6B	Group 13B	Group 20B	Group 27B	Group 34B
48 hour	Group 7B	Group 14B	Group 21B	Group 28B	Group 35B
72 hour	Group 8B	Group 15B	Group 22B	Group 29B	Group 36B
Group 1: Frozen squid rings, Groups B: Cooked + marinated frozen squid rings					

2.2. Methods

2.2.1. Chemical Analyses

Fatty acid analysis

The determination of fatty acid methyl esters (FAME) analysis was done by Ege University Laboratory in Ege Matal according to the method of COI/T.20/Doc. No 33 for olive oils method. In this method; a total of 37 component mixture of FAME (Supelco) identifying the fatty acids was chosen as the standard for retention time. Area ratio, which was under the relevant peak, was used for the quantitative analysis. Additionally, the Agilent 6890 GC-FID system was used for FAME analysis. In addition to this the column was an Agilent HP-88 capillary column (100 m x 0.25 mm ID x 0.2 μm) and the split ratio was 1:100. Further, injection and detector temperatures were 250°C and

260°C, respectively. The temperature program was as follows; in the first process; the oven temperature was held at 120°C for 1 min and then in the second process, this temperature increased to 240°C at a rate of 4°C/min and hold for 5 min (International Olive Oil, 2017).

2.2.2. Preparation of the samples

Free aminoacid

Samples were weighed according to their concentration. It was dissolved in some water and then 1 ml of SSA was added. Further, it was completed to 100 ml. Samples were centrifuged at high speed. If necessary, it was filtered and transferred to 1 ml. SSA=2.5 g 5-Sulfosalicylic acid dihydrate was dissolved in 25 ml of water.

Total aminoacid

0.2-0.5 g of sample (homogenized) was weighed and then 5 ml of 6 N HCl was added to it. 250µl 2mM phenol was added to prevent oxidation. 0.1 g of Na2SO3 was added to optimize the recovery of cystine, methionine, and tyrosine. It was kept for 24 hours at 110 degrees. The pH of the sample was set near the neutral (6.7-7.3). Further, it was completed with water to 100 ml. In addition to this, it was centrifuged for 5 min at 4000 rpm. If it was necessary filtered and 1 ml was added into the viale. The device method was made using the following application (Agilent Technologies, Santa Clara, CA, USA).

2.3. Statistical Analysis

In the study, model experiments were conducted using curve estimation in Social Sciences Statistics Package (SPSS) Version 25.0 software. Linear and quadratic regression models were used according to the method (Montgomery and Runger, 2003).

3. RESULTS

The results of the fatty acid and free amino acid profiles of marinated and un-marinated frozen squid rings (Group A) are shown in Table 3-5.

The results of fatty acid and free aminoacid profiles of cooked and cooked marinated frozen squid rings (Group B) are given in Table 6-8.

Tablo 3. Fatty acid results in group A (%)

Groups	Lauric	Myristic	Pentadecanoic	Cis-10-Pentadecanoic	Palmitic	Heptadecanoic	Stearic	Elaidic	Oleic	Linolelaidic	Linoleic	Arachidic	t-Linolenic	Linolenic	cis-8,11,14-eicosatrienoic	Ericic	cis-13,16-docosadienoic	Lignoceric	cis-5,8,11,14,17-eicosapentaenoic	cis-4,7,10,13,16,19-docosahexaenoic
1	nd	nd	nd	nd	17.04	nd	6.02	nd	nd	nd	nd	nd	nd	6.41	nd	nd	12.01	nd	nd	58.31
2A	nd	nd	nd	nd	15.49	nd	9.35	6.60	nd	nd	5.89	nd	nd	7.30	nd	5.02	10.01	nd	nd	40.34
3A	nd	nd	nd	nd	14.34	nd	10.56	nd	nd	nd	nd	nd	nd	17.39	nd	nd	10.33	nd	nd	47.37
4A	nd	nd	nd	nd	14.22	nd	15.92	nd	nd	nd	12.99	nd	nd	11.33	nd	nd	20.47	nd	nd	25.07
5A	nd	nd	nd	nd	17.83	nd	10.25	nd	8.26	nd	9.96	nd	nd	7.97	nd	nd	11.29	nd	7.68	26.76
6A	nd	7.14	8.49	nd	12.06	8.39	nd	7.32	nd	8.63	nd	nd	8.10	nd	nd	nd	13.69	nd	10.18	16.00
7A	nd	nd	nd	nd	33.48	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	28.32	nd	nd	38.20
8A	nd	nd	nd	nd	26.74	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	21.88	nd	18.57	32.81
9A	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
10A	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
11A	nd	nd	nd	nd	19.15	nd	7.34	nd	nd	nd	nd	nd	nd	7.39	nd	nd	12.96	5.61	nd	47.55
12A	nd	nd	nd	nd	19.86	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	13.21	nd	nd	66.94
13A	6.79	nd	nd	nd	21.94	nd	3.55	nd	nd	nd	nd	nd	nd	3.13	nd	nd	17.57	nd	nd	47.03
14A	nd	nd	nd	nd	21.61	1.87	6.63	nd	1.89	nd	nd	nd	nd	5.41	nd	1.84	12.72	4.50	nd	43.54
15A	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	62.10	nd	nd	37.90
16A	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
17A	nd	nd	nd	nd	24.70	nd	7.40	4.48	nd	nd	nd	nd	nd	7.17	nd	5.22	12.84	7.78	nd	30.41
18A	nd	nd	nd	nd	28.00	nd	8.51	nd	nd	nd	nd	nd	nd	6.72	nd	nd	13.93	10.44	nd	32.42
19A	nd	nd	nd	nd	68.96	nd	nd	nd	nd	nd	nd	31.04	nd	nd	nd	nd	nd	nd	nd	nd
20A	nd	nd	nd	16.93	13.22	nd	19.70	nd	nd	nd	nd	nd	nd	6.76	nd	nd	8.89	8.71	nd	25.77
21A	nd	nd	nd	nd	30.40	nd	8.57	nd	nd	nd	nd	nd	nd	7.17	nd	nd	15.10	7.12	nd	31.64
22A	nd	nd	nd	19.26	7.40	nd	10.62	nd	nd	nd	nd	nd	nd	6.21	nd	nd	10.59	8.52	nd	37.40
23A	nd	nd	nd	nd	28.65	nd	9.00	nd	nd	nd	nd	nd	nd	8.68	nd	nd	15.09	7.01	nd	31.58
24A	nd	nd	nd	nd	23.75	nd	7.12	8.00	nd	nd	3.17	nd	nd	6.08	nd	nd	12.16	6.03	nd	33.70
25A	nd	nd	nd	nd	26.55	nd	8.24	5.46	nd	nd	nd	nd	nd	6.48	nd	nd	15.86	7.07	nd	30.34

26A	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
27A	nd	nd	nd	20.84	6.01	nd	11.43	nd	nd	nd	nd	nd	nd	5.56	nd	nd	12.66	10.46	nd	33.04
28A	nd	nd	nd	nd	25.76	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	30.14	nd	nd	44.10
29A	nd	nd	nd	nd	32.28	nd	9.50	nd	nd	nd	nd	nd	nd	6.97	nd	nd	15.71	7.13	nd	28.40
30A	nd	nd	nd	nd	25.79	nd	8.00	3.38	nd	nd	nd	nd	nd	6.80	nd	nd	15.20	4.28	nd	36.56
31A	nd	nd	nd	16.70	7.78	nd	8.89	nd	nd	nd	nd	nd	nd	nd	9.62	nd	10.09	13.72	nd	33.20
32A	nd	nd	nd	22.60	10.31	6.52	13.03	nd	nd	nd	nd	nd	nd	6.11	nd	nd	11.21	7.85	nd	22.38
33A	nd	nd	nd	17.39	nd	nd	19.72	nd	nd	nd	nd	nd	nd	nd	nd	nd	44.59	nd	nd	18.30
34A	nd	nd	nd	nd	25.58	nd	8.18	nd	nd	nd	nd	nd	nd	6.45	nd	nd	15.44	8.61	nd	35.73
35A	7.62	7.39	nd	12.87	7.47	6.84	10.28	nd	6.45	nd	nd	nd	nd	nd	nd	nd	6.90	12.19	nd	22.00
36A	nd	nd	nd	nd	20.66	nd	16.31	nd	nd	nd	nd	nd	nd	12.94	nd	nd	16.39	nd	nd	33.70

Group 1: control group. Butyric, Caproic, Capric, Caprylic, Undecaonic, Tridecanoic, Cis-10-Heptadecanoic, Heneicosanoic, cis-11,14-eicosadienoic, cis-11-eicosenoic, Behenic, cis-11,14,17-eicosatrienoic, Arachidonic, Myristoleic, Nervonic, Palmitoleic, Tricosanoic weren't found.

Table 4. Free amino acids results in group A (kg/mg)

Group	Aspartic acid	Glutamic acid	Serine	Asparagine	Glutamine	Histidine	Glycine	Threonine	Arginine	Alanine	Tyrosine	Cystine	Valine	Methionine	Norvaline	Phenylalanine	İsoleucine	Leucine	Lysine	Hdroxyproline
1	0.18	3.92	nd	1.18	nd	nd	6.07	0.99	0.92	0.28	0.5	0.21	0.28	nd	nd	nd	nd	0.27	0.26	nd
2A	1.18	3.38	nd	0.74	nd	nd	5.82	1.08	0.86	nd	0.34	0.33	0.17	nd	nd	nd	nd	nd	nd	nd
3A	0.2	2.71	nd	1.2	0.16	nd	5.61	0.75	1.03	0.29	0.61	nd	nd	nd	nd	nd	nd	0.53	0.38	nd
4A	0.18	3.95	nd	0.87	nd	nd	5.33	1.28	0.97	nd	0.64	0.59	nd	0.48	nd	nd	0.57	0.82	0.86	nd
5A	0.16	3.31	nd	0.99	nd	nd	6.5	1.47	1.37	nd	0.66	0.22	0.19	nd	nd	nd	nd	0.38	nd	nd
6A	0.58	3.08	nd	1.45	nd	nd	8.47	nd	0.96	0.72	0.64	0.4	0.41	0.53	nd	nd	nd	1.04	0.68	nd
7A	0.2	3.39	nd	0.98	nd	nd	5.77	0.98	1.02	1.12	0.47	0.69	nd	nd	nd	nd	nd	0.38	0.14	nd
8A	0.45	3.29	nd	0.8	nd	nd	5.83	0.81	0.63	nd	0.29	0.15	0.28	nd	nd	0.2	nd	0.45	0.57	nd
9A	nd	nd	nd	0.37	nd	nd	2.08	nd	0.26	0.4	0.31	0.32	0.33	nd	nd	nd	nd	nd	nd	nd
10A	0.14	0.13	nd	nd	nd	nd	1.87	nd	nd	0.3	0.27	0.28	0.22	nd	nd	nd	nd	nd	nd	nd
11A	0.22	nd	nd	nd	nd	nd	1.65	nd	nd	0.23	0.29	0.1	0.17	nd	nd	nd	nd	nd	nd	nd
12A	0.17	4.02	nd	nd	nd	nd	1.41	nd	1.19	1.14	0.25	0.12	0.15	nd	nd	nd	nd	nd	nd	nd
13A	0.17	3.88	nd	0.1	nd	nd	1.13	0.13	0.22	0.25	0.3	0.15	0.22	nd	nd	0.14	nd	0.18	nd	nd

14A	0.12	4.14	nd	0.23	nd	nd	0.92	nd	0.2	nd	0.28	0.42	0.11	nd	nd	nd	nd	nd	nd	nd
15A	0.12	3.62	nd	nd	nd	nd	1.27	nd	0.36	0.33	0.28	0.17	0.29	nd	nd	0.2	nd	0.11	nd	nd
16A	nd	3.88	nd	0.13	nd	nd	0.63	nd	0.23	0.24	0.25	0.14	0.19	nd	nd	0.17	nd	nd	nd	nd
17A	0.12	3.72	nd	0.23	nd	nd	0.77	nd	0.16	0.19	0.4	0.27	0.16	nd	nd	0.14	nd	nd	nd	nd
18A	nd	5.84	0.26	nd	0.85	nd	nd	nd	nd	nd	0.13	0.28	0.4	nd	0.91	nd	nd	nd	nd	nd
19A	0.28	4.21	nd	0.25	nd	nd	0.25	0.45	nd	0.19	0.27	nd	0.29	nd	nd	nd	nd	nd	nd	nd
20A	0.12	4.48	nd	0.13	nd	nd	nd	0.29	nd	nd	nd	nd	0.21	nd	nd	nd	nd	nd	nd	0.28
21A	0.17	5.01	nd	0.17	nd	nd	0.4	0.12	nd	nd	0.14	nd	0.21	nd	nd	nd	nd	nd	nd	0.29
22A	13	4.83	nd	0.17	nd	nd	nd	0.41	0.1	nd	0.16	nd	0.3	nd	nd	nd	nd	nd	nd	nd
23A	nd	4.23	nd	0.29	nd	nd	nd	0.43	nd	nd	0.17	nd	0.27	nd	nd	nd	nd	nd	nd	0.35
24A	nd	4.21	0.13	nd	0.11	0.27	0.12	nd	nd	nd	nd	0.19	0.22	nd	nd	nd	nd	nd	nd	nd
25A	nd	4.15	nd	nd	nd	0.66	0.3	0.2	nd	nd	0.15	nd	0.2	nd	nd	nd	nd	nd	nd	0.24
26A	nd	4.38	nd	0.2	nd	0.72	0.29	0.29	nd	nd	0.29	nd	0.26	nd	nd	nd	nd	nd	nd	0.3
27A	nd	4.51	nd	0.14	nd	nd	0.18	0.22	0.62	nd	0.17	nd	3.97	nd	nd	nd	nd	nd	nd	0.18
28A	nd	4.2	nd	0.11	nd	0.62	0.22	0.19	nd	nd	0.11	0.12	0.38	nd	nd	nd	nd	nd	nd	0.42
29A	nd	3.67	nd	0.13	nd	nd	0.32	0.16	0.73	0.1	0.18	nd	0.35	nd	nd	nd	nd	nd	nd	0.15
30A	1.18	3.79	nd	0.21	nd	nd	nd	0.34	nd	nd	0.18	nd	0.35	nd	nd	nd	nd	nd	nd	nd
31A	nd	4.09	nd	nd	nd	nd	nd	0.33	nd	nd	0.19	nd	0.36	nd	nd	nd	nd	nd	nd	nd
32A	0.13	4.02	nd	nd	nd	nd	nd	0.29	nd	nd	0.15	nd	0.33	nd	nd	nd	nd	nd	nd	nd
33A	nd	3.78	nd	nd	nd	nd	nd	0.12	0.29	nd	nd	nd	0.25	nd	nd	nd	nd	nd	nd	nd
34A	nd	4.15	nd	0.13	nd	nd	nd	0.12	0.28	nd	0.12	nd	0.22	nd	nd	nd	nd	nd	nd	nd
35A	nd	3.67	nd	nd	nd	nd	nd	0.2	nd	nd	0.15	nd	0.3	nd	nd	nd	nd	nd	nd	nd
36A	nd	3.95	nd	nd	nd	nd	nd	0.13	0.3	nd	0.16	nd	0.31	nd	nd	nd	nd	nd	nd	nd

Trptophan wasn’t found. Group 1: control group

Table 5. Hidrolysis results in group A (kg/mg)

GROUPS	Aspartic acid	Glutamic acid	Asparagine	Glutamine	Histidine	Glycine	Threonine	Arginine	Alanine	Tyrosine	Cystine	Valine	Methionine	Norvaline	Trptophan	Phenylalanine	İsoleucine	Leucine	Lysine	Hdroxyproline	Sarcosine	Proline
1	0	1.92	2.66	86.36	18.37	0.5	0	0	9.82	10.01	3.04	0	1.78	1.92	6.01	5.05	3.49	2.03	35.08	0	114.39	0
2A	0	0.98	2.37	1596	64.77	0.02	0	0	24.66	1.05	1.16	0	1.08	0	16.03	5.15	5.09	5.2	0	0	0	0
3A	0	1.12	3.23	1545.83	66.85	0.01	0	40.23	6.37	0.09	0.44	11.37	1.64	0	36.41	5.29	8.71	26.53	0	88.49	0	0
4A	0	1.1	2.45	1063.59	48.99	0	0	29.25	1.95	0.09	0.44	6.99	1.87	0	13.64	4.34	8.44	16.03	0	60.25	0	0
5A	0	0.91	2.04	919.52	37.82	0	0	24.03	1.08	0.07	0.92	2.77	1.1	0	29.1	3.81	8.32	7.39	0	54.15	0	0
6A	0	0.86	2.43	1037	45.34	0.01	0	26.71	1.67	0.07	2.41	2.08	0	0	89.21	5.26	0	9.68	0	53.58	0	0
7A	0	0.9	2.7	1060.21	85.64	0.18	0	29.29	3.22	0.09	3.54	3.73	0	0	97.81	5.9	0	18.78	0	68.01	0	0
8A	0	0.71	2.67	1085.5	115.62	4.73	0	24.35	3.31	0.09	3.48	4.17	1.55	0	98.36	4.33	9.18	19	0	49.27	0	0
9A	0.22	1.54	2.51	1508.58	74.46	0.14	0	11.92	4.29	0.15	5.62	6.46	0	0	106.65	4.9	7	3.23	0.8	25.29	0	0
10A	0.14	1.58	1.86	1215.01	45.32	0.01	0	9.52	3.17	0.1	3.86	3.53	0.92	26.02	59.47	4.99	5.38	1.88	1.58	0	0	0
11A	0.12	1.51	2.72	1760.5	73.11	0.26	0	13.13	4.24	0.09	5.52	6.87	0.88	0	75.24	5.67	6.07	2.59	1.23	0	0	0
12A	0.09	1.45	2.91	1561.18	90.26	2.39	0	13.26	3.55	0.07	5.52	9.83	0.71	0	85.89	5.27	5.81	5.64	0.32	17.15	0	60.71
13A	0.09	1.59	3.02	1472.66	94.69	2.54	0	15.36	3.89	0.08	5.67	10.67	0.62	0	88.11	6.66	0	13.67	0	26.55	0	0
14A	0.08	1.73	2.73	1659.63	90.43	2.39	0	13.85	3.51	0.1	5.16	9.25	0.61	0	74.83	7.62	0	10.74	0	19.13	0	0
15A	0.07	1.69	2.24	1796.67	82.77	1.3	0	11.19	2.56	0.09	5.5	8.68	0.61	0	76.14	7.54	0	10.86	0	16.78	0	0
16A	0.08	1.65	3.45	2724.56	131.4	2.8	0	17.94	4.46	0.13	10.18	15.14	0.66	0	161.67	10.7	0	21.61	0	20.94	0	0
17A	0.08	1.76	5.43	3730.03	193.2	8.57	0	22.82	10.01	0.16	13.83	19.6	0.71	0	226.43	12.84	0	29.82	0	25	0	0
18A	0.09	1.84	5.97	4011.8	203.9	8.58	0	24.43	11.76	0.17	14.2	19.33	0.74	0	229.79	12.89	0	30.46	0	38.82	0	0
19A	0.56	5.67	0.92	0	0	0	0	2.27	0.43	0.07	2.31	3.99	0.63	0	0	0	0	0	0.94	8.76	0	20.43
20A	0.7	12.76	0.17	0	0	0	0	0	0	0.04	0.38	1.04	0.9	0	0	0	0	0	0.14	0	0	0
21A	0	5.21	1.53	0	21.51	0	0.24	0	0	1.82	1.8	1.29	7.95	0.04	61.43	3.81	0	0.88	0	0	0	0
22A	0	1.77	8.09	5547.85	317.34	0	0	0	73.29	2.13	11.09	4.73	4.53	0	303.68	5.32	14.91	39.15	0	42.65	0	0
23A	0.39	1.01	9.09	4781.85	309.8	7.38	0	45.86	19.64	0.11	18.35	26.5	0.61	0	349.69	6.97	13.79	40.53	0	40.9	0	202.83
24A	0.21	1.1	8.68	3691.86	256.44	8.03	0	37.12	12.65	0.15	14.66	24.31	0.63	0	271.35	6.47	11.96	19.75	0	41.27	0	164.71
25A	0.13	1.18	5.1	2223.29	156.4	4.34	0	17.58	5.17	0.1	9.39	7.04	0.57	0	157.41	4.78	7.74	6.64	0	25.85	0	91.42
26A	0.11	1.09	5.13	2349.7	175.69	6.01	35.88	24.86	5.26	0.11	8.85	6.59	0.59	0	145.35	4.88	7.07	5.72	0	20.27	0	0
27A	2.56	2.43	0	0	0	0	30.24	30.91	0.61	0.12	3.91	0	0	0	10.37	5.21	0	0.4	0	0	0	0

28A	91.31	3.29	0	0	0	0	0	18.87	0.43	0.08	2.23	0	0	21.39	8.56	8.2	0	0.08	2.51	0	0	0
29A	99.55	7.92	4.29	1726.24	173.51	37.3	0	10.47	1.9	0.05	2.15	0	0	35.18	69.57	10.77	3.54	0.07	1.52	0	0	0
30A	144.37	42.01	8.38	2199.61	240.09	49.71	0	14.98	2.94	0.04	2.6	1.01	0	49.84	99.5	13.93	2.39	0.02	1.92	0	0	0
31A	193.32	46.26	9.37	3083.03	351.95	70.4	0	20.13	4.55	0.05	3.37	1.08	0	72.43	143.69	18.05	2.44	0.02	3.38	0	0	0
32A	132.05	35.63	7.25	2158.49	265.57	47.02	0	14.51	3.26	0.05	2.78	1	0	58.55	115.74	14.85	2.34	0.02	2.52	0	0	0
33A	32.78	35.71	9.84	1993.33	247.67	35.14	0	14.21	3.9	0.08	2.71	1.05	0	52.8	115.5	14.12	2.32	0.02	1.94	0	0	0
34A	115.31	36.94	10.6	2023.46	231.34	34.58	0	13.1	3.98	0.08	2.77	1.06	0	0	99.42	12.95	2.3	0.02	2.51	0	0	0
35A	110.51	36.81	9.98	2105.55	216.36	48.12	0	12.9	3.76	0.05	2.9	1.08	0	0	108.56	13.11	2.31	0.02	2.18	0	0	0
36A	109.15	34.88	7.48	1973.16	188.68	43.63	0	12.3	2.82	0.06	2.42	1.16	0	44.35	107.05	13.06	2.32	0.02	1.57	0	0	0
Group 1: control group.																						

Tablo 6. Fatty acid results in group B (%)

Groups	**Caprylic**	**Lauric**	**Myristic**	**Myristoleic**	**Cis-10-Penta**	**Palmitic**	**Palmitoleic**	**Heptadecanoic**	**Stearic**	**Elaidic**	**Oleic**	**Linoleic**	**cis-11-eicose**	**Linolenic**	**Ericic**	**cis-13,16-docos**	**Lignoceric**	**cis-5,8,11 14,17**	**Nervonic**	**cis-4,7,10 ,13,16**
1	nd	nd	nd	nd	nd	17.04	nd	nd	6.02	nd	nd	nd	nd	6.41	nd	12.01	nd	nd	nd	58.31
2B	nd	nd	nd	nd	nd	22.02	nd	nd	6.94	nd	4.70	nd	nd	5.97	nd	12.36	5.66	nd	nd	42.35
3B	nd	nd	nd	nd	nd	23.20	nd	3.14	7.41	nd	nd	nd	nd	5.63	nd	12.95	nd	nd	nd	47.68
4B	nd	nd	nd	nd	nd	20.24	nd	3.18	6.97	nd	2.96	nd	nd	6.18	3.43	12.35	7.05	nd	nd	37.64
5B	nd	nd	nd	nd	nd	21.43	nd	2.90	7.34	nd	nd	nd	nd	5.80	3.95	12.81	5.97	nd	nd	39.81
6B	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7B	nd	nd	nd	nd	nd	21.94	nd	nd	8.70	nd	nd	nd	nd	7.23	nd	11.67	nd	nd	nd	50.46
8B	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	28.69	nd	nd	nd	71.31
9B	nd	nd	nd	nd	nd	15.76	nd	nd	6.70	nd	nd	nd	nd	7.70	nd	9.45	nd	9.42	nd	50.97
10B	nd	nd	nd	nd	20.85	nd	nd	6.12	6.13	5.84	nd	nd	5.69	nd	5.38	11.89	nd	nd	nd	38.12
11B	nd	nd	5.04	nd	nd	21.70	nd	5.44	6.52	4.33	nd	nd	nd	5.57	4.84	10.36	nd	nd	nd	36.18
12B	nd	nd	nd	nd	15.87	nd	nd	5.79	4.77	5.14	nd	nd	nd	3.44	6.31	9.38	nd	nd	nd	49.29
13B	nd	nd	nd	nd	nd	16.94	nd	nd	4.56	4.07	nd	3.27	nd	4.02	4.00	14.97	nd	nd	nd	48.17
14B	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
15B	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
16B	3.93	nd	4.53	nd	14.58	nd	3.51	nd	3.92	2.43	nd	nd	3.92	nd	3.11	8.06	5.58	nd	nd	46.43
17B	nd	nd	nd	nd	26.80	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	17.32	nd	nd	nd	55.88

18B	nd	nd	5.55	nd	13.52	nd	nd	nd	8.59	nd	nd	nd	nd	11.38	nd	9.98	7.18	nd	nd	43.79
19B	nd	nd	nd	nd	14.29	nd	nd	nd	5.60	nd	nd	nd	28.35	nd	nd	8.15	nd	nd	nd	43.61
20B	nd	2.96	3.17	nd	14.20	7.02	nd	3.58	10.29	nd	nd	nd	3.74	5.32	3.30	9.01	nd	nd	nd	37.41
21B	nd	3.98	6.00	nd	13.80	7.43	nd	5.74	11.58	3.49	nd	nd	nd	3.75	4.50	8.41	7.78	nd	nd	23.55
22B	nd	4.58	3.95	nd	15.24	5.78	nd	5.03	8.71	nd	5.63	nd	3.93	4.38	nd	9.28	4.73	nd	nd	28.75
23B	nd	nd	1.87	1.34	24.87	nd	1.84	nd	6.68	2.21	1.91	nd	5.72	nd	1.86	12.49	nd	nd	nd	39.22
24B	nd	nd	nd	nd	19.79	nd	nd	3.20	5.43	5.91	5.57	nd	5.15	nd	4.24	10.97	nd	4.68	nd	35.07
25B	nd	nd	2.00	nd	24.70	nd	nd	nd	6.89	nd	nd	nd	5.98	nd	4.84	12.98	2.26	nd	nd	40.35
26B	nd	nd	5.36	nd	nd	17.78	nd	3.70	4.20	nd	4.20	nd	nd	4.72	5.08	nd	10.58	nd	6.91	37.47
27B	nd	nd	3.31	nd	19.08	nd	3.70	nd	5.51	2.80	2.58	3.53	4.94	nd	4.78	10.00	7.95	nd	nd	31.82
28B	nd	nd	nd	nd	20.47	nd	4.70	nd	5.69	5.50	5.84	nd	4.86	nd	6.85	10.37	nd	nd	nd	35.72
29B	nd	nd	4.70	nd	15.13	nd	5.50	nd	5.23	nd	nd	nd	10.53	nd	5.04	9.87	nd	nd	nd	44.01
30B	nd	nd	2.39	nd	21.09	nd	nd	nd	5.23	3.65	2.87	nd	4.85	nd	2.19	11.56	6.37	nd	nd	39.80
31B	nd	nd	8.02	nd	29.55	nd	nd	nd	8.37	7.33	nd	nd	6.04	nd	6.68	11.04	nd	nd	nd	22.98
32B	nd	nd	2.07	1.88	22.67	nd	nd	1.76	6.71	3.25	3.36	nd	4.85	nd	3.70	10.77	3.17	nd	nd	35.80
33B	nd	nd	4.92	nd	17.84	nd	nd	nd	7.10	5.39	5.14	nd	5.19	nd	5.07	10.85	5.02	nd	nd	33.48
34B	nd	nd	4.27	nd	19.07	nd	nd	nd	5.96	4.74	5.35	nd	5.20	nd	4.04	12.17	5.22	nd	nd	33.98
35B	nd	nd	4.69	nd	21.78	nd	nd	nd	5.67	3.31	4.30	nd	5.17	nd	3.46	10.93	4.39	nd	nd	36.31
36B	nd	nd	nd	nd	13.29	nd	nd	nd	12.17	nd	nd	nd	14.72	nd	12.39	20.03	nd	nd	nd	27.40
Arachidic, Butyric, Caproic, Capric, Pentadecanoic, Undecaonic, Tridecanoic, t-Linolenic, Linolelaidic, Cis-10-Heptadecanoic, Heneicosanoic, cis-8,11,14-eicosatrienoic, cis-11,14-eicosadienoic, Behenic, cis-11,14,17-eicosatrienoic, Arachidonic, Tricosanoic weren't found. Group 1: control group.																			

Table 7. Free amino acids results in group B (kg/mg)

Groups	Aspartic acid	Glutamic acid	Serine	Asparagine	Histidine	Glycine	Threonine	Arginine	Alanine	Tyrosine	Cystine	Valine	Lysine
1	0.18	3.92	nd	1.18	nd	6.07	0.99	0.92	0.28	0.5	0.21	0.28	0.26
2B	0.35	3.53	nd	1.12	nd	5.79	nd	0.7	nd	0.45	0.34	0.17	nd
3B	0.21	3.67	nd	1.19	nd	5.47	0.82	1.2	0.91	0.42	0.49	0.2	nd
4B	0.11	3.12	nd	0.91	nd	5	nd	0.49	0.19	0.39	0.29	0.34	nd
5B	0.24	3.43	nd	0.6	nd	3.95	0.8	0.89	0.17	0.45	0.23	0.12	nd
6B	0.38	3.75	nd	0.9	nd	4.2	nd	0.9	nd	0.65	0.33	0.18	nd
7B	0.14	2.87	0.57	nd	nd	2.55	0.57	0.53	0.15	0.62	0.28	0.12	0.13
8B	nd	4.02	0.53	0.33	1.21	nd	nd	1.2	nd	0.63	0.23	0.38	nd
9B	nd	4.12	nd	nd	nd	0.11	0.25	nd	nd	0.14	0.17	0.26	nd

10B	nd	3.85	nd	nd	nd	nd	0.14	22	nd	nd	nd	0.15	nd
11B	nd	42	nd	0.11	nd	nd	nd	0.33	nd	0.12	nd	0.27	nd
12B	nd	34.35	nd	nd	nd	nd	0.21	0.21	nd	0.25	nd	0.28	nd
13B	nd	3.94	nd	nd	nd	0.21	0.32	nd	nd	0.21	nd	0.28	nd
14B	nd	3.51	nd	0.15	nd	nd	nd	0.23	0.11	nd	nd	0.31	nd
15B	nd	3.32	nd	nd	nd	nd	nd	0.21	nd	nd	0.24	0.34	nd
16B	nd	3.38	nd	nd	nd	nd	nd	0.35	nd	nd	nd	0.5	nd
17B	nd	3.05	nd	nd	nd	nd	nd	0.23	nd	0.25	nd	0.43	nd
18B	nd	3.57	nd	nd	nd	nd	0.34	nd	nd	0.48	nd	0.49	nd
19B	nd	3.22	nd	nd	nd	nd	0.24	nd	nd	nd	0.26	0.25	nd
20B	nd	3.04	nd	nd	nd	nd	0.32	nd	nd	0.13	nd	0.24	nd
21B	nd	3.16	nd	nd	nd	nd	0.24	nd	nd	0.19	0.18	0.2	nd
22B	nd	3.27	nd	nd	nd	nd	0.39	nd	nd	0.1	nd	0.34	nd
23B	nd	2.96	nd	nd	nd	nd	0.27	nd	nd	0.13	0.21	0.34	nd
24B	nd	3.1	nd	nd	nd	nd	0.26	nd	nd	0.26	nd	0.22	nd
25B	nd	3.06	nd	nd	nd	nd	0.25	nd	nd	0.12	0.35	0.25	nd
26B	nd	3.88	nd	nd	nd	nd	0.22	nd	nd	0.12	0.19	0.28	nd
27B	nd	3.17	nd	nd	nd	nd	0.2	nd	nd	0.17	nd	0.25	nd
28B	nd	3.71	nd	nd	nd	nd	0.17	nd	nd	0.18	0.29	0.31	nd
29B	nd	3.54	nd	nd	nd	0.11	0.17	0.1	nd	0.2	0.42	0.25	nd
30B	nd	3.43	nd	nd	nd	nd	0.23	nd	nd	0.2	0.2	0.26	nd
31B	nd	3.39	nd	nd	nd	nd	0.15	nd	nd	0.12	0.15	0.19	nd
32B	nd	3.89	nd	nd	nd	nd	0.21	nd	nd	0.1	0.26	0.23	nd
33B	nd	3.79	nd	nd	nd	nd	0.17	nd	nd	nd	0.22	0.24	nd
34B	nd	3.85	nd	nd	nd	nd	0.16	0.1	nd	0.14	0.12	0.24	nd
35B	nd	4.08	nd	nd	nd	nd	0.21	nd	nd	0.26	0.19	0.21	nd
36B	nd	4.01	nd	nd	nd	nd	0.18	0.11	nd	nd	0.16	0.27	nd
Glutamine, Methionine, Norvaline, Trptophan, Phenylalanine, İsoleucine, Leucine, Hdroxyproline weren't found. Group 1: control group													

Table 8. Hidrolysis results in group B (kg/mg)

Group	Aspartic acid	Glutamic acid	Asparagine	Glutamine	Histidine	Glycine	Threonine	Arginine	Alanine	Tyrosine	Cystine	Valine	Methionine	Norvaline	Trptophan	Phenylalanine	İsoleucine	Leucine	Lysine	Hdroxyproline	Sarcosine	Proline
1	0	1.92	2.66	86.36	18.37	0.5	0	0	9.82	10.01	3.04	0	1.78	1.92	6.01	5.05	3.49	2.03	35.08	0	114.39	0
2B	0	0.61	2.38	921.45	88.77	3.29	0	15.41	2.46	0	2.55	3.43	1.75	0	70.33	3.43	6.55	14.03	0	18.23	0	0
3B	0	0.6	2.52	1162.16	85.58	2.46	0	17.42	2.88	0	2.71	2.47	1.75	0	63.02	3.22	6.08	10.41	0	24.16	0	0
4B	0	0.6	2.61	1688.12	87.59	2.06	0	23.48	3.51	0	2.92	2.21	1.83	0	68.66	3.87	6.51	9.51	0	27.97	0	0
5B	0	0.59	3.22	1838.44	109.76	3.19	0	28.37	4.86	0	4.59	3.37	1.91	0	88.17	4.73	6.57	12.87	0	28.75	0	0
6B	0	0.66	3.38	0	0	0.81	0	32.03	12.42	0	2.22	9.35	3.74	0	110.94	4.92	6.32	13.75	0	28.28	0	0
7B	0	0.82	2.29	0	0	0.4	0	28.55	7.25	0.42	1.63	8.74	0	0.28	98.18	4.31	6.01	10.79	0	15.63	0	0
8B	0.38	1.15	2.71	1757.71	96.67	2.03	0	20.63	4.1	0.11	6.7	7.64	0	0.42	122.5	4.67	7.69	8.3	0.96	19.53	0	0
9B	93.79	31.67	5.32	2109.69	174.86	35.7	0	12.29	2.5	0.08	2.13	1.41	0	56.48	98.56	12.53	2.27	0.02	1.79	0	0	0
10B	103.33	33.09	5.84	2261.18	174.42	32.72	0	13.91	2.85	0.07	2.4	1.38	0	61.47	115.2	13.58	2.29	0.02	2.52	0	0	0
11B	17.89	3.59	0.45	288.03	3.49	0	0	2.38	0.28	0	1.11	1.13	0	0	8.16	5.08	2.35	0.01	0	0	0	0
12B	2.85	0.46	0.06	0	0	0	0	0	0.04	0	0.47	0.94	0.62	0.44	0.68	1.85	0	0.01	0	0	0	0
13B	1.69	0.48	0.15	0	0	0	0.04	0	0.04	0.03	0.48	0.8	0.57	0	1.16	1.85	0	0.02	0	0	0	0
14B	1.63	1.43	0.1	0	0	0	0.02	0.24	0	0	0.56	0.87	0.7	0	2.08	1.74	0	0.02	0.51	0	0	0
15B	2.53	4.68	0.05	0	0	0	0	0	0	0	0.55	0.91	0.69	0.41	1.55	1.66	0	0.02	0.54	0.92	0	0
16B	2.64	1.13	0.06	0	0	0	0.01	0	0	0.04	0.53	0.83	0.62	0.31	1.46	1.72	0	0.02	0.76	0	0	182.31
17B	2.75	1.29	0.07	0	0	0	0.02	0	0	0.03	0.51	0.81	0.58	0.31	0.33	1.71	0	0.02	0.39	0	0	0
18B	2.65	6.01	0.07	0	0	0	0.01	0	0	0.02	0.51	0.84	0.55	0.32	0.3	1.67	0	0.02	0.26	0	0	0
19B	2.62	6.01	0.07	0	0	0	0.02	0	0	0.02	0.53	0.84	0.56	0.31	1.73	1.72	0	0.02	0	0	0	6.08
20B	3.22	5.82	0.06	0	0	0	0.02	0	0.04	0	0.54	0.82	0.55	0.33	2.58	1.83	0	0	0	0	0	4.09
21B	0.77	1.22	0.18	0	0	0	0.01	0.19	0	0	0.55	0.8	0.53	0.29	4.01	1.96	0	0	0.41	0	0	0
22B	0.66	0.25	0.19	0	0	0	0.01	0.19	0	0.03	0.54	0	0	0.27	3.5	1.87	2.18	0	0.47	0	0	0
23B	2.94	0.24	0.06	0	0	0	0.01	0.25	0.03	0.03	0.52	0	0	0.27	1.31	1.64	0	0	0	0	0	0
24B	2.89	0.23	0.06	0	0	0	0.01	0.25	0.02	0.03	0.52	0.79	0	0.33	1.13	1.64	0	0	0	0	62.4	0
25B	2.73	0.22	0.05	0	0	0	0.01	0.21	0.01	0.03	0.48	0.76	0.53	0.35	0.32	1.69	0	0.01	0	0	121.68	0
26B	2.79	1.11	0.05	0	0	0	0.01	0.25	0	0.03	0.49	0	0.53	0.33	0.44	1.7	0	0.02	0.56	0	202.8	3.41

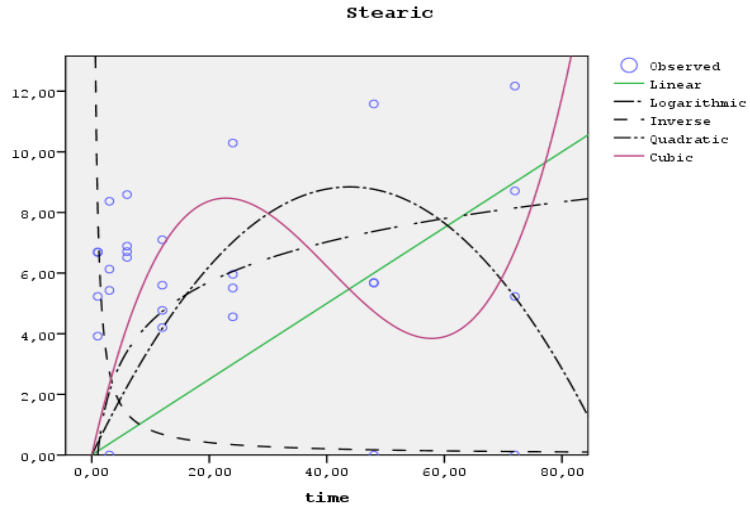
27B	2.9	6.22	0.05	0	0	0	0.01	0.27	0	0.03	0.53	0	0.53	0.31	4.43	1.75	2.71	0.02	0	0	260	3.02
28B	2.9	6.51	0.05	0	0	0	0.01	0.28	0	0.02	0.55	0	0	0.24	4.59	1.75	2.18	0.02	0	0	276	4.03
29B	2.82	6.53	0.05	0	0	0	0	0.25	0	0.02	0.56	0	0	0.24	4.8	1.71	0	0.02	0	0	386.4	8.06
30B	2.78	6.51	0.06	0	0	0	0	0.23	0	0.03	0.52	0.76	0.55	0.29	4.61	1.66	0	0.02	0	0	296.8	10.41
31B	2.79	6.6	0.05	0	0	0	0	0.36	0.02	0.03	0.53	0.76	0.56	0.29	4.23	1.68	0	0.02	0	0	275.6	4.98
32B	2.94	6.63	0.04	0	0	0	0	0	0.01	0	0.53	0.8	0.58	0	0	1.69	0	0	0	0	239.2	4.33
33B	2.91	6.62	0.05	0	0	0	0	0	0.02	0	0.5	0.8	0.61	0	0	1.67	0	0	0.85	0	176.64	6.5
34B	2.82	6.35	0.05	0	0	0	0	0	0	0.02	0.51	0	0.68	0	0.51	1.67	0	0	0.4	0	161.28	0
35B	2.91	6.62	0.05	0.05	0	0	0.01	0	0	0.02	0.53	0	0.64	0	0.32	1.65	0	0	0.44	0	184.8	0
36B	2.81	7.29	0.05	0.01	0	0	0	0	0	0.03	0.48	0	0	0.34	0.37	1.66	0	0.02	0	0	145.2	11.76

Group 1:Control group.

In the study; the marination process increased the aspartic acid, glutamic acid, asparagine, histidine, glycine, norvaline, tryptohane, and phenylalanine concentrations of the marinated squid samples, whereas the concentrations of methionine, isoleucine, leucine and hydroxyproline decreased. On the other hand, the marination process caused to increase the aspartic acid, glutamic acid, sarcosine, and proline concentrations of the cooked squid rings, whereas the marination process caused to decrease the concentrations of glutamine, histidine, glycine, arginine, alanine, cystine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, hydroxyproline.

According to the results of statistical analyses; since fatty or amino acid values could not be determined in most trials in un-marinated and marinated squid rings (Group A), it was not possible to examine how the marination time would affect the fatty or amino acid values in this study. Therefore, it was investigated how the marination time would affect the increase or decrease of the fatty acid and amino acid ratios in cooked marinated squid rings (Group B). For this purpose, fatty acid and amino acid ratios were determined for cooked squid rings marinating at different times (1, 3, 6, 12, 24, 48, 72 hours). To see the time-dependent changes of each amino acid and fatty acid, the graphs were drawn and given in Figure 1.

Figure 1. Various model fit graphs to time-amino acid or time-fatty acid relationship: (a) Stearic–Time, (b) cis-13,16-docosadienoic – Time, (c) cis-4,7,10,13,16,19-docosahexaenoic – Time, (d) Valine– Time, (e) Cystine – Time, (f) Phenylalanine – Time



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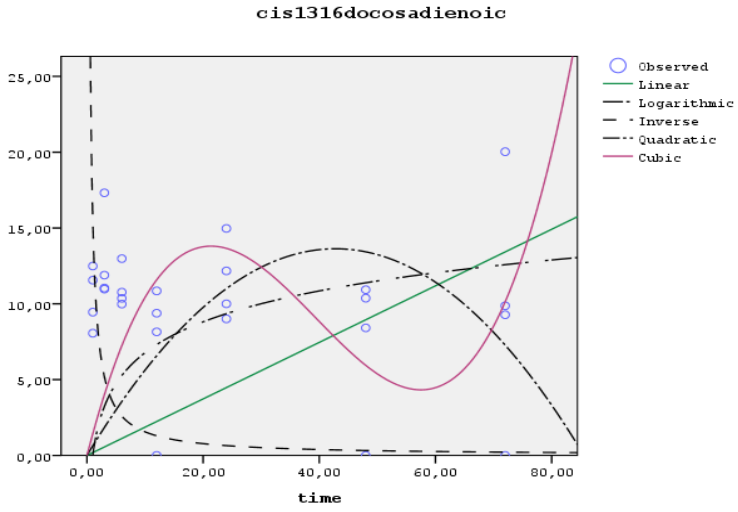

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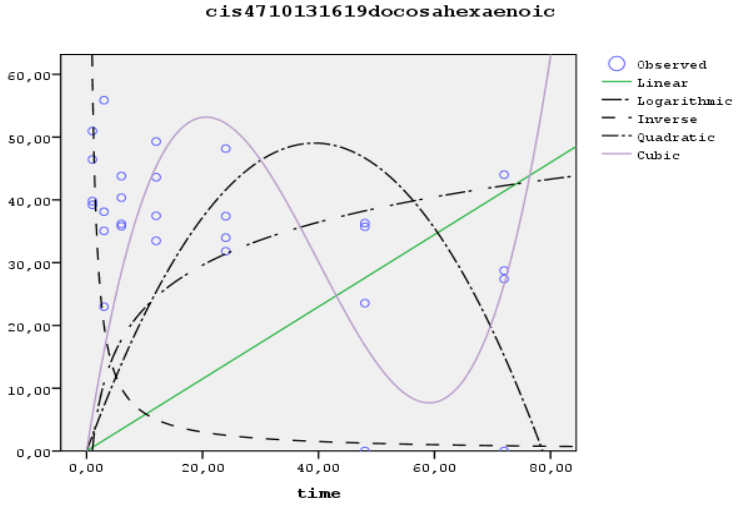
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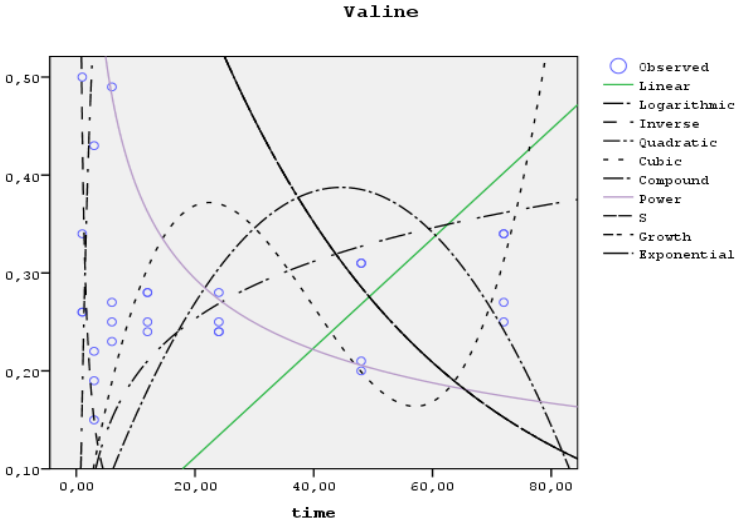
(c)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Growth
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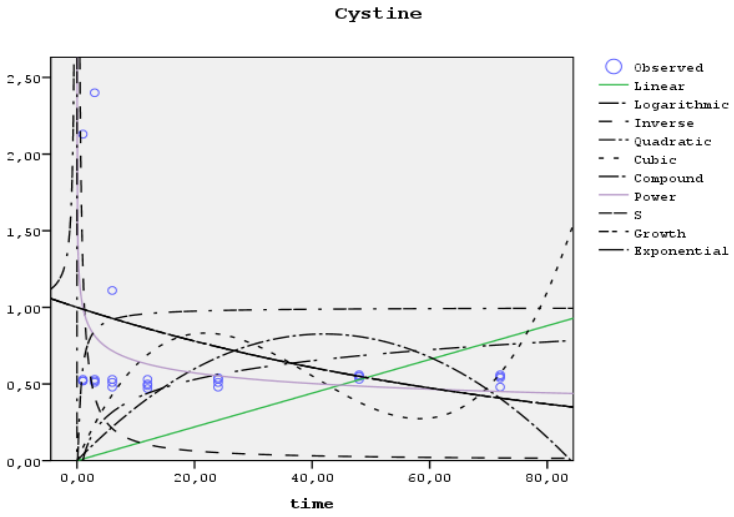
(d)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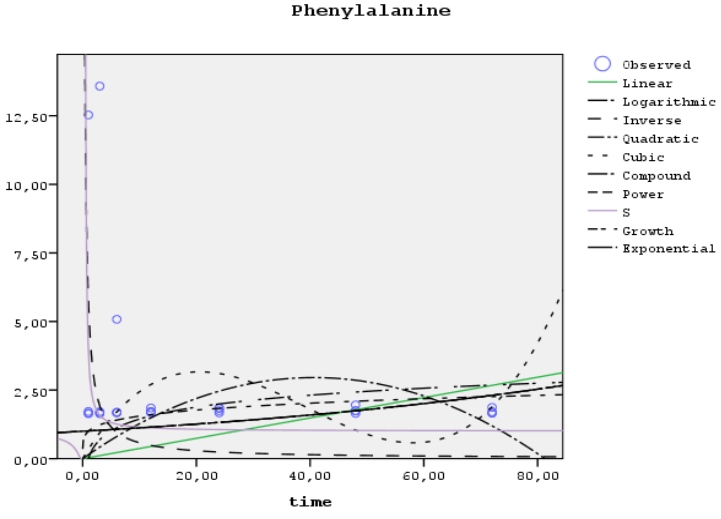
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Growth
Exponential

(f)

With the help of these graphs, it will be convenient to adhere to various regression models to determine the number of amino acids or fatty acids depending on time. For this reason, some regression models were created between the two variables by taking the fatty acid or amino acid values as the dependent variable (Y) and by taking the time as the independent variable (t). Model experiments were conducted using curve estimation in the Social Sciences Statistics Package (SPSS) Version 25.0 software. There are many model options in the curve estimation such as linear, logarithmic, inverse, quadratic, cubic, power, S-curve model etc. Model equations that are significant and used in this study are given below:

$$Y = \beta_0 + \beta_1 t + \epsilon \quad \text{Linear model} \quad (1)$$

$$Y = \beta_0 + \beta_1 t + \beta_2 t^2 + \beta_3 t^3 + \epsilon \quad \text{Cubic model} \quad (2)$$

$$lnY = ln\beta_0 + \beta_1 \ln(t) + \epsilon \quad \text{Power model} \quad (3)$$

$$lnY = \beta_0 + \frac{\beta_1}{t} + \epsilon \quad \text{S – curve model} \quad (4)$$

In these models β_0, β_1, β_2 and β_3 represent the unknown parameters of the model, and ϵ represents the error term of the model. Details on the subject are given in (Montgomery and Runger, 2003). After various model trials, it was seen that the constant term was not statistically significant. For this reason, there is no constant term in the prediction models. The linear estimation equations for each fatty acid or amino acid for which a meaningful result were obtained were determined and given in Table 9. For the amino acid or fatty acids of interest, the most resonable estimation equations were determined as an alternative to linear models and were given in Table 10. Here, while determining the most appropriate equation, the model in which the model coefficients are significant and the adjusted r^2 value is the largest is taken into consideration.

Table 9. Linear regression models by fatty or amino acid values of the cooked marinated squid rings

		r	r^2	$Adjr^2$	p-value
Stearic	$\hat{Y} = 0.125t$	0.644	0.415	0.393	0.000
cis-13,16-docosadienoic	$\hat{Y} = 0.186t$	0.587	0.345	0.321	0.001
cis-4,7,10,13,16,19-docosahexaenoic	$\hat{Y} = 0.575t$	0.524	0.274	0.247	0.004
Valine	$\hat{Y} = 0.006t$	0.656	0.430	0.409	0.000
Cystine	$\hat{Y} = 0.011t$	0.467	0.218	0.189	0.011
Phenylalanine	$\hat{Y} = 0.037t$	0.321	0.103	0.07	0.09

Table 10. Appropriate regression models by fatty or amino acid values of the cooked marinated squid rings

	Appropriate Model	Estimated regression equation	r	r^2	$Adjr^2$	p-value of the coefficients		
						β_1	β_2	β_3
Stearic	Cubic model	$\hat{Y} = 0.855t - 0.026t^2 + 0{,}000216t^3$	0.824	0.678	0.640	0.000	0.002	0.008
cis-13,16-docosadienoic	Cubic model	$\hat{Y} = 1.476t - 0.047t^2 + 0.000401t^3$	0.798	0.636	0.593	0.001	0.001	0.005
cis-4,7,10,13,16,19-docosahexaenoic	Cubic model	$\hat{Y} = 5.842t - 0.191t^2 + 0.002t^3$	0.831	0.691	0.654	0.000	0.000	0.001
Valine	Power model	$ln\hat{Y} = -0.4085\ln(t)$	0.853	0.728	0.718	0.000		
Cystine	Power model	$ln\hat{Y} = -0.1862\ln(t)$	0.787	0.619	0.605	0.000		
Phenylalanine	S – curve model	$ln\hat{Y} = \frac{1.3818}{t}$	0.613	0.376	0.353	0.000		

In the first column of these tables, the prediction equations for fatty or amino acid values of the cooked marinated squid rings were given. These equations were used to estimate the fatty or amino acid value by time. In the next three columns, the values of r, r^2, and $Adjr^2$ were given. The r^2 value represents the portion of the fatty or amino acid values that can be explained by the model. For example; $r^2 = 0.42$ for stearic fatty acid. In other words, the value of stearic fatty acid can be explained by 42% by the linear model. However, there was an unexplained 58% in the model for the estimation of the value of this fatty acid. This result hints that the model can still be improved and different variables can be used for fatty acid estimation. When there was more than one model for the same variable, the value $Adjr^2$ was used. Since alternative model resultsare given here, it can be decided which of the two models is preferred by using $Adjr^2$ values. For example, for stearic fatty acid, $Adjr^2$ value of the linear model was 0.393, and the $Adjr^2$ value of the cubic model was 0.640. Therefore, the cubic model here was a more suitable model than the linear model that could be used for the prediction of stearic fatty acid.

Using estimated regression equation (1) stearic fatty acid values can be estimated by time. The p values in the last column of Table 9 show the significance level obtained in the hypothesis test to determine whether the β_1 coefficient is significant or not. The p values in the last three columns of Table 10, on the other hand, show the significance levels obtained in the hypothesis test to determine whether the β_1, β_2, and β_3 coefficients are significant, respectively.

First of all, for each amino acids and fatty acids, linear regression equations which are the most basic and simple regression models are given in Table 9. In addition, the most appropriate prediction equation for each amino acid and fatty acid is given in Table 10. Therefore, it would be appropriate to estimate the amount of amino acids or fatty acids depending on time for each fatty acid and amino acid using the equations in Table 10. Some calculations were made for various hour values and prediction values for each fatty acid and amino acid are given in the Table 11.

Table 11. Estimated fatty acid and amino acid values depending on time

t	1	3	6	12	18	24	36	48	60	72	75	80
$\hat{Y}_{Ste}$	0.829	2.337	4.241	6.889	8.226	8.530	7.162	5.024	4.356	7.398	9	12.592
$\hat{Y}_{cis-13}$	1.429	4.016	7.251	11.637	13.679	13.895	10.933	6.907	5.976	12.296	15.497	22.592
$\hat{Y}_{cis-4,7}$	5.653	15.861	28.608	46.056	54.936	57.84	56.0888	61.536	94.92	176.976	207.525	268.96
$\hat{Y}_{Val}$	1	0.638	0.481	0.362	0.307	0.273	0.231	0.206	0.188	0.174	0.171	0.167
$\hat{Y}_{Cys}$	1	0.815	0.716	0.630	0.583	0.553	0.513	0.486	0.467	0.451	0.448	0.442
$\hat{Y}_{Phen}$	3.982	1.585	1.259	1.122	1.080	1.059	1.039	1.029	1.023	1.019	1.019	1.017

4. DISCUSSION

In one study (Kilinc and Cakli, 2005); the increase in glutamic acid and aspartic acid concentrations of frozen fillet of sardine (*Sardina pilchardus*) after the marination process were reported. The effects of storage on the quality of smoked fish (*Oreochromis nilotius*) were examined by Aveloja et al. (2015). The authors of this study, frozen *O.niloticus* reported that the amino acid decreased with the increasing storage period. In addition, glutamic acid and lysine were explained as the most concentrated essential amino acid (EAA), and non-essential amino acid (NEEA) respectively (Aveloja et al., 2015). Changes in the composition of amino acids in the muscle of marinated fish during storage were investigated by the author (Ozden, 2005), that aspartic acid, threonine, proline, glycine, tyrosine, and lysine concentrations in marinated products greatly influenced their quality. The results obtained according to the above studies (Kilinc and Cakli, 2005; Ozden, 2005; Aveloja et al., 2015) stated that the effects of the marinating process were observed in changes in amino acid values of squid rings.

Fatty acids have been considered very important owing to their health benefits and their nutritional values (Asmaa et al., 2015). Total SFA of marinated anchovy and rainbow trout increased during storage, whereas PUFA concentrations decreased significantly in only marinated anchovy (Ozden, 2005). The fatty acid contents of squid meat increased for MUFA, PUFA, and SFA. The fatty acid content of squid meat increased for MUFA, PUFA, and SFA. This increase in fatty acids can be caused by various mechanisms that

occur during heating processes, such as thermal hydrolysis, deactivation of enzymes, loss of essential fatty acids, water loss, lipid oxidation, fatty acid migration or transformation (Sutikno et al., 2019). The authors (Topuz, 2016) idenfied that the reduced concentration of acetic acid and salt slightly increased the proportion of omega-3 fatty acids, but there was no significant effect were determined on the omega-3 ratio of marinated little tunny fish (*Euthynnus alletteratus*) fillets. In another report (ElShehawy and Farag, 2019); PUFA percentage ranged between 22.6% and 69.4%, of which linoleic acid recorded 66.3% in canned tuna and 34.4% in canned sardine, whereas (C22:2) was 10.8% in canned mackerel. In addition, the nutritional value (PUFA / SFA) of canned tuna samples was higher compared to canned sardines and canned mackerel samples (ElShehawy and Farag, 2019). In one report; the major EAA in the frozen squid samples were lysine, arginine, and leucine, while the major NEAA were aspartic acid, glutamic acid and glycine. On the other hand, DHA, and EPA contents, EAA, and NEAA values were significantly lower in frozen squid compared to fresh samples ($P<0.05$) (Gabr, 2010). In another study; the highest EAA in paper squid (*Loligo edulis*) was reported as arginine containing 6.24% and the lowest EAA in paper squid was histidine, which was found as 1.43%. In addition to this, the highest NEAA in paper squid was determined as glutamic acid, which was 12.13% and the lowest NEAA in paper squid was tyrosine, which was 2.49% (Toyohara et al., 1999).

The authors (Anggo et al., 2015) studied the amino acid composition of fish paste obtained from anchovy (*Stolephorus sp*.). The authors indicated in this report that the contents of lysine, glutamic acid, leucine, aspartic acid and arginine were explained as higher than the other amino acids. The EFA determined was linolenic acid, which was higher at the beginning period of the fermentation process. During the fermentation period; most of values of free amino acids were increased. However, almost all fatty acids decreased except for the EPA, DHA, and stearic acid. In the study, amino acid compositions of squid rings were determined that the marination process increased in the glutamic acid, aspartic acid, asparagine, histidine, glycine, norvaline, tryptohan, and phenylalanine concentrations of the squid samples. Additionally, the marination process caused in increase the aspartic acid, glutamic acid, sarcosine, and proline concentrations of cooked squid rings. However, palmitic and linolenic acids decreased in cooked squid rings after the

marination process. Our results were well correlated with the above studies (Ozden, 2005; Gabr, 2010; Anggo et al., 2015; Topuz, 2016; Loppies et al., 2021) that the marination and cooking processes affected the changes in some of the fatty acid and amino acid values.

5. CONCLUSION

In the study; the marination and cooking processes effected the values of the fatty and aminoacid profiles of squid rings. The marination process caused to increase the asparagine, aspartic acid, norvaline, glutamic acid, histidine, glycine, tryptophan and phenylalanine concentrations of squid samples, whereas it given rise to decrease the concentrations of the methionine, isoleucine, leucine, and hydroxyproline. Conversely, when compared cooked squid rings with cooked marinated squid rings, the marination process caused to increase in the glutamic acid, aspartic acid, sarcosine, and proline concentrations, whereas the marination process caused to decrease in the concentrations of glutamine, histidine, glycine, arginine, alanine, cystine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, hydroxyproline, palmitic and linolenic acids. Appropriate regression mathematical models were created to predict the amino acid and fatty acid values of squid rings. According to the results of mathematical statistical models, statistically significant prediction models were found for some of the fatty acid or amino acid values of cooked marinated squid rings.

As a result; the cubic model for stearic ($Adjr^2 = 0.64$), cis-13,16-docosadienoic ($Adjr^2 = 0.593$) and cis-4,7,10,13,16,19-docosahexaenoic ($Adjr^2 = 0.654$), the power model for valine ($Adjr^2 = 0.718$) and cystine ($Adjr^2 = 0.605$), and the S-curve model for phenylalanine ($Adjr^2 = 0.353$) were determined the most suitable models to predict the estimated values of cooked marinated squid rings.

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CHAPTER 7

TEMPERATURE LIMITATION IN SPIRULINA PLATENSIS OUTDOOR CULTURE

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1. INTRODUCTION

Spirulina platensis is a photoautotrophic prokaryote that naturally inhabits alkaline lakes in tropical and subtropical climates. The optimum living temperature for *Spirulina* is around 35°C. Cyanobacteria *Spirulina platensis* has attracted the attention of human beings and has commercial value with its protein content up to 70%, phycocyanin, chlorophyll *a* pigments, mineral and vitamin richness, especially high iron content. Today, it is cultivated in ponds or photobioreactors in many countries. *Spirulina* culture medium is enriched with nutritive elements and high levels of carbonate and bicarbonates, similar to the nutrient composition of the lakes where it naturally lives, and its pH value is maintained around 9-10.

The microalgae *Spirulina* is a good source of protein and can be used directly as food. However, due to the valuable metabolites it contains, it is also used as a food additive, food colorant, soil fertilizer and cosmetics industry. *Spirulina* can be a good feed additive in poultry feeding and fish feeding.There are many studies on the use of *Spirulina* in nutritional and health applications in the field of health. Researches are carried out on the protective against cancer, viruses and microorganisms effects of *Spirulina*, which are attributed to the content of endonuclease (which repair damaged DNA), calcium sulfated polysaccharide (which inhibits in vitro replication of viruses) and fatty acids (especially high amount of linolenic acid), respectively. The metallo-protective role of beta carotene, vitamins C, E, superoxide dismutase enzyme, selenium and blue pigment phycocyanin are attributed to phycocyanin. (Seyede *et al*., 2013).

Spirulina has a stimulating effect on the human immune system as well as antihypertensive, anticancer, antioxidant and antibacterial, anti-inflammatory properties (Hirata *et al*, 201; Wu *et al*, 2016; Ovando, 2018; Pham *et al*., 2016). *Spirulina* contains powerful antioxidants and fights free radicals (Gad *et al*, 2011; Yu *et al*, 2016; Belay, 2002) and has antimicrobial activity (Sun *et al*, 2015) and has the advantage of natural food preservatives (Sun *et al*, 2015; Ovando *et al*, 2018).

Microalgae, which produces these valuable metabolites that are desired to be consumed by humans, which have economic value, especially *Spirulina*, need to be cultured in large volumes, and this has to be outdoors, not in the laboratory environment.

The main physical factors affecting biomass efficiency in microalgae out cultures are light and temperature. In outdoor cultures, the difference between day and night temperature averages and the range of sunshine hours during the day affect photosynthesis efficiency.

2. OPTIMUM TEMPERATURE

The temperature of 30-35 °C is the optimal temperature range for *Spirulina* growth.Outside the tropics, the growth of *Spirulina* in outdoor ponds during winter is very slow, unable to grow, resulting in low products (Richmond, 1992). For this reason, regions where the temperature drops below 15 °C in winter are not suitable for *Spirulina* culture. (Richmond, *et al*., 1990). Algae producers often cover ponds with a transparent polyethylene cover to maintain optimum culture conditions, reduce production cost, keep the high temperature and maintain on cold but sunny days and reduce the contamination risk (Vonshak, 1992).

It is known that in mass production of algae, every 10 °C increase in culture temperature means that the use of nutrients approximately doubles. Biomass efficiency in large volumes of outdoor algae cultures can be improved by estimating light and temperature factors. (Grobbelaar *et al.*, 1990).

It is known that the cell composition and physiological state of phytoplankton are greatly influenced by temperature, which is one of the leading physical environmental conditions (Torzillo and Vonshak, 1994), especially altered fatty acids (FA) metabolism, and vitamin and carotenoid content in cells (Borowitzka, 1988; Becker, 1993; Olguin, *et al*., 2001).

Gamma linolenic acid (C18:3), which is evaluated as a pharmaceutical and nutraceutical substance, is found in Spirulina in fairly high quantities (Cohen *et al*., 1987). Another important factor is pH, which affects the physiology of algae by determining the solubility of carbon dioxide and minerals in the environment (Becker, 1994). Light is another environmental factor that affects cell composition.

The main biological factor affecting the efficiency of *Spirulina* biomass is temperature, and daily and seasonal temperature changes are an important factor in biomass production.As noted by Vonshak and Richmond (1988), since the daily and seasonal temperature values will not always be optimal for *Spirulina*, it is difficult to achieve and maintain maximum biomass productivity

in outdoor cultures. Indeed, in desert climates or similar regions, even in summer, if the culture temperature in the morning is 10 °C below the optimum values, this will prevent the full use of the photosynthetic capacity of the culture in the first hours of the morning.

In a study comparing the efficiency of *Spirulina* in outdoor ponds and photobioreactors, better results in photobioreactors were attributed to the partial preservation of heat in the photobioreactor. (Torzillo *et al*., 1986). In studies carried out under controlled conditions, it was determined that the maximum biomass from *Spirulina* was taken at 30-35 °C (Tomaselli *et al*., 1987).

It has been seen that it is important to maintain the optimum temperature as much as possible throughout the day in order to increase the *Spirulina* biomass. The night time temperature of the culture represents another important factor that can affect the net biomass productivity as it influences the loss of organic matter at night due to respiration. Studies on microalgae grown both in vitro and outdoors have shown that the total loss of biomass in the twelve hours of darkness is 2-10% of the biomass before entering the dark hours (Grobbelaar and Soeder, 1985).

In outdoor *Spirulina* cultures, nocturnal biomass loss measurements showed that as much as 35% of the biomass produced during the day is losted at night .

A study was carried out to investigate the effect of temperature on yield and nocturnal biomass loss in outdoor culture of *Spirulina platensis* in a tubular photobioreactor. These results showed that the amount of net product in *Spirulina* outdoor culture depends on photosynthetic production during the day and biomass loss in the dark at night. The results showed that when *Spirulina* is grown at optimum day and night temperature and at optimum biomass concentration, the total night loss reaches 4-6% of dry matter, at the end of the day in summer. The loss during the night period is 10-16% of the daytime production (Vonshak and Richmond, 1988).

In a study we carried out, in which *Spirulina platensis* was cultured in a greenhouse in the winter season in a subtropical climate, low temperature growth was observed.

Spirulina platensis was obtained from Israel, and cultivated in the *Spirulina* medium (Schlösser, 1982) which contains 18.6 g $NaHCO_3$, 8.06 g Na_2CO_3, 1.00g K_2HPO_4, 5.00 g $NaNO_3$, 2.00g K_2SO_4, 2.00 g NaCl, 0.40 g

$MgSO_4.7H_2O$, 0.02g $CaCl_2$. $2H_2O$, 0.02g $FeSO_4.7H_2O$, 0.16g $EDTANa_2$, 10.0 ml of microelement solution were added per 1 Liter. The microelement solution is 0.001g $ZnSO_4.7H_2O$, 0.002g $MnSO_4.7H_2O$, 0.01g H_3BO_3, 0.001g $Na_2MoO_4.2H_2O$, 0.001g $Co(NO_3)2.6H_2O$, 0.00005g $CuSO_4.5H_2O$, 0.7g $FeSO_4.7H_2O$, 0.8g $EDTANa_2$ It is prepared to be in 1L.

Temperature, pH and OD measurements were made in the morning and afternoon, daily between 27 January and 10 March 2020 in the *Spirulina* culture ponds in the subtropical climate around the Iskenderun Bay in the Eastern Mediterranean. Measurements were taken from a *Spirulina* culture pond measuring 1m x 5m x 0.20 m in the greenhouse. The culture in the pond was mixed continuously at a speed of 20-30 cm sec^{-1} with the pedal system. The starter cultures used to inoculate the ponds were developed in vitro.

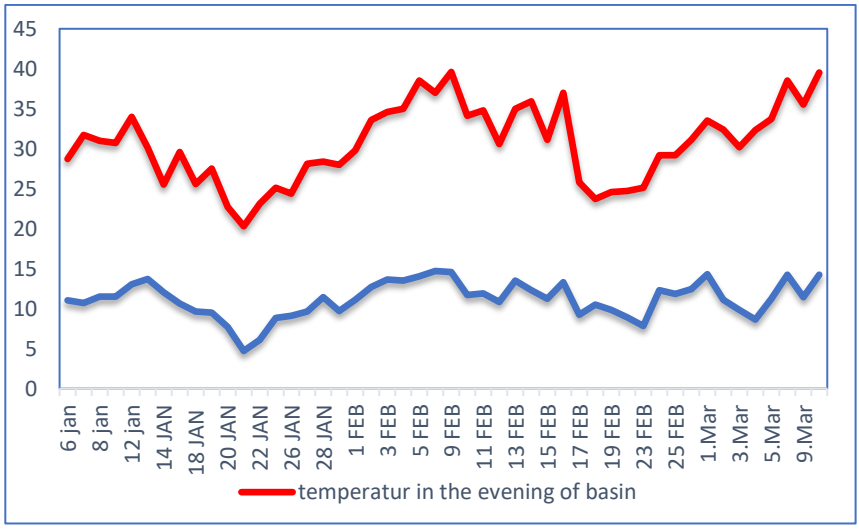

Figure 1. Morning and afternoon temperature values of the *Spirulina* culture in the pond.

With the knowledge that the optimum temperature range for *Spirulina platensis* life is 33-35 °C, it has been determined that the average day temperature in the morning and evening is 15.3 °C, and it is seen that it is approximately 20 °C lower than the optimum growth temperature for *Spirulina* (Figure 1).

According to the OD data (Figure 2) determined in the morning and evening between January and March, when temperatures are approximately 20 °C lower than the optimum culture temperature for *Spirulina*, the growth curve was formed as above.

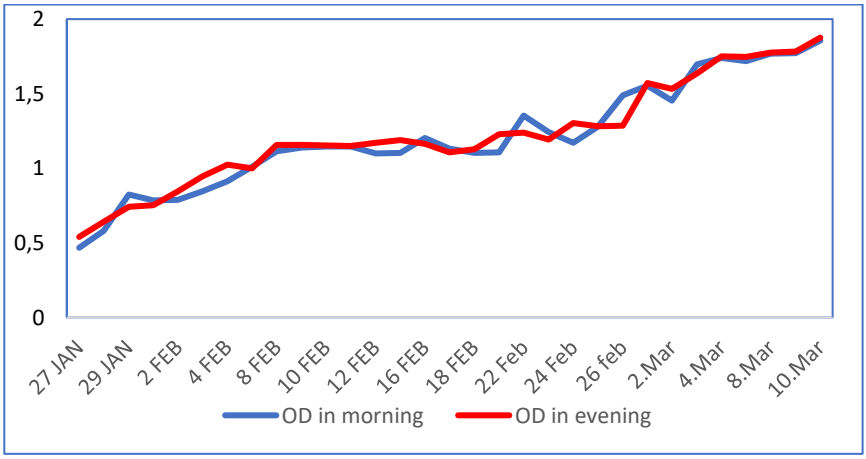

Figure 2. Morning and afternoon OD values of the *Spirulina* culture in the pond.

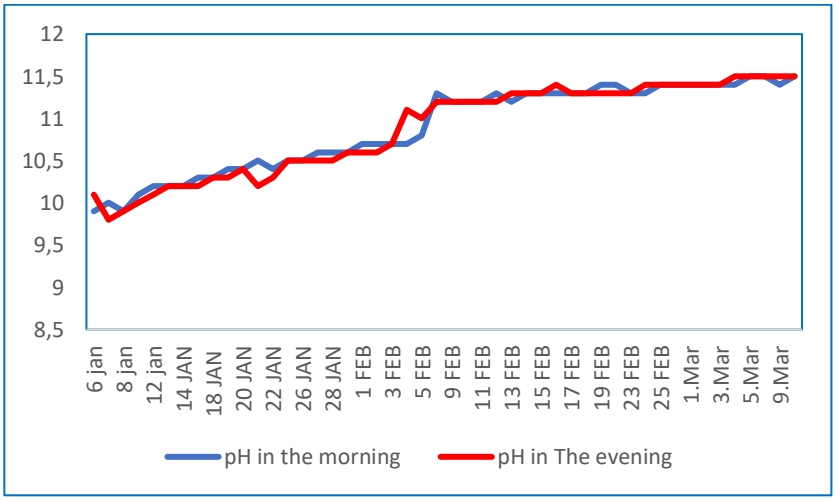

Figure 3. Morning and afternoon pH values of the *Spirulina* culture in the pond.

Spirulina platensis is known to be an alkalophilic type of algae and is known to grow best in alkaline waters, waters with a pH of 9 and above. As

seen in the graph (Figure 3), pH values remained between 9.9 and 11.5 during the day in winter conditions. Perhaps the best thing about keeping the pH high despite low temperatures is that the risk of contamination is still low. Because the removal of *Spirulina* under relatively optimum conditions may be an opportunity for another organism.

Considering that the night temperature value is determined as 4.7 °C and there is a difference of 8 °C between evening and morning temperatures, it is seen that the cells continue their lives, albeit slowly, even though they are far from the optimum degrees. Of course, when evaluated economically, the slow growth in question is not a desirable situation, but considering that the temperature will rise towards the first spring and summer as of March 9, it can be said that the winter period is a tolerable period.

While the optimum temperature for the growth of *Spirulina* is around 35-38 °C, limited growth takes place around 15 °C. Production in outdoor pools is mostly limited by temperature.

The response to temperature changes involves synthesis or degradation, depending on changes in the intensity and quality of the light. In outdoor cultures, the combination of high oxygen concentration and low temperature can be seen at the onset of the cold season. If the culture temperature drops well below optimum but still lighting is sufficient, it is sufficient to sustain photosynthesis at a significant rate. Such conditions can be very common in desert areas where the morning temperature of the culture is well below optimum and the light intensity is high enough to induce photoinhibition (Vonshak and Torzillo, 2004).

Outdoor algae cultures are exposed to changes in environmental conditions. These changes occur on two different time scales. One is the circadian cycle, which includes the change in light and temperature over a 24-hour cycle. The other is a seasonal cycle that changes according to the climatic and geographic location of the habitat where the algae grow.

The diurnal variation in overall productivity, day and night temperature differences, has not yet been adequately investigated in Spirulina cultures, even during the peak production period in summer. The decrease in nighttime temperature may affect photosynthetic productivity in the early morning hours (Richmond *et al*., 1990), whereas nocturnal biomass loss may be less if the

daytime temperature continues throughout the night (Torzillo *et al.*, 1991; Vonshak *et al*., 1982).

Another problem with low winter temperatures is the difficulty of maintaining healthy cultures in open outdoor ponds to be used as inoculum cultures for the next growing season. Under cold winter temperatures, the culture often deteriorates and precipitates quickly. Therefore, vaccine cultures must be scaled up in limited volumes in ponds within the greenhouse, or vaccine cultures must be made at laboratory scale, which will be a slow and expensive process.

3. PROTEIN AND AMINO ACID CONTENT OF *SPIRULINA*

In the study, the effect of temperature on crude protein and carbohydrate content of *Spirulina platensis* mass harvested at the end of the sunny period was investigated (Table 1). During the study, two reactors were used from May to September, the temperature was set at 35±1°C in one reactor and 25±1°C in the other (Torzillo *et al.*, 1991).

Table 1. Influence of temperature on crude protein and carbohydrate contents of *Spirulina platensis* biomass harvested at the end of the daylight period .

Temperature	**25°C**		**35** °C	
Month	**Protein**	**Carbohydrate**	**Protein**	**Carbohydrate**
May	59.58	28.17	66.76	22.75
June	57.00	28.83	65.34	22.40
July	60.00	27.18	64.45	24.58
August	60.98	24.32	66.71	20.81
September	62.26	23.24	67.85	17.98

(Torzillo *et al.*, 1991).

Spirulina protein has a balanced composition of amino acids, with concentrations of methionine, tryptophan and other amino acids almost similar to those of casein although this depends upon the culture media used (Table 2).

Table 2. Amino acid composition of *Spirulina* (g/100 g)

Source	Siam Algae Co. Ltd., Thailand	IPGSR Malaysia
Lysine	2.60–3.30	4.63±0.07
Phenylalanine	2.60–3.30	4.10±0.08
Tyrosine	2.60–3.30	3.42±0.10
Leucine	5.90–6.50	8.37±0.13
Methionine	1.30–2.00	2.75±0.05
Glutamic acid	7.30–9.50	7.04±0.14
Aspartic acid	5.20–6.00	5.37±0.11
Tryptophan	1.00–1.60	1.98±0.05
Cystine	0.50–0.70	0.6±0.03
Serine	3.84±0.06	
Arginine	4.94±0.07	
Histidine	2.81±0.06	
Threonine	3.35±0.06	
Proline	4.11±0.05	
Valine	4.02±0.06	
Isoleucine	3.85±0.10	
Alanine	10.81±0.14	
Glycine	6.66±0.10	

(FAO, 2021)

In the study we carried out about subtropical climate the effect of temperature on protein and amino acid composition of *Spirulina platensis* was investigated. The experiments were carried out in four fiberglass ponds, 1m^3 capacity for each, in the greenhouse in summer (July) and winter (January). The cultures were circulated by the paddle wheels at a flow rate about 20 cm s^{-1}

continuously. Culture depth was maintained at 10 cm in the ponds. The cultures were grown in *Spirulina* medium.

The mean day temperatures were recorded as 33.9±0.4 ºC and 18.6±0.5 ºC, the mean night temperatures were recorded to be 29.9±0.2 ºC and 14.4±0.2 ºC in summer and winter, respectively. While the growth in the cultures reached the maximum in 9 days in summer (July), the maximum growth was reached in 38 days in the winter (January).

In the study conducted in subtropical climate, the effect of temperature on protein and amino acid composition of *Spirulina platensis* was investigated. Mean daytime temperatures in summer and winter months were recorded as 33.9±0.4 ºC and 18.6±0.5 ºC, and nighttime temperatures as 29.9±0.2 ºC and 14.4±0.2 ºC, respectively. While the growth in the cultures reached its maximum in 9 days in summer (July), the maximum growth was reached in 38 days in winter (January).

While the mean pH values in *Spirulina* cultures were recorded as 9.25 and 9.52 for summer and winter, respectively, filament densities were determined as $123x10^3$ and 94.10^3 in July and January, respectively. The highest dry matter amounts were determined as 1375 (mg/L) for July and January. While the protein contents (%) in summer and winter months were determined as 72.9±0.3 and 33.16±0.2, respectively, amino acid contents are as follows. Protein analysis was done with wet biomass.

Amino acids concentrations of *S. platensis* (mg/100 g wet samples) grown in summer and winter are shown in Table 3.

Table 3. Amino acids composition of *S. platensis* (mg/100 g wet samples) grown in summer and winter

Amino Acids (mg/100 g)	Summer	Winter
Threonine	2009.7	1106.5
Valine	2381.7	1491.4
Methionine	551.6	318
Isoleucine	2261.1	1311.9
Leucine	3198.5	1862.3
Phenylalanine	1716.6	1043.2
Lysine	2280.3	1105.3
Aspartic acid	3791.3	1889.8
Serine	1869.4	907.9
Glutamic acid	6747.4	3782.2
Glycine	2163.2	1210.2
Alanine	2179.3	1774.8
Tyrosine	1121.5	871.6
Histidine	818.5	471.6
Proline -	465.2	
Cystine -	117.2	
Arginine -	1153.5	
Total	33.09	20.88

(Uslu, *et al*., 2009).

While the average daytime temperature is 18.6 °C in the winter period, the average night temperature is recorded as 14.4 °C, and the optimum growth temperature for *Spirulina* is seen to be quite far from 33-35 °C. In the study, the end of the logarithmic phase was reached in 38 days. The long time for product production, low productivity in the biomass, low protein content, and

the absence of amino acids Proline, Cystine and Arginine in the biomass in winter are associated with low temperature (Uslu *et al*., 2009).

Torzillo *et al*. (1984) observed that the protein content of *Spirulina* was at the highest level in summer, depending on environmental factors, especially temperature and light intensity.

Tomaselli *et al*. (1988) studied the effect of temperature on *Spirulina platensis* M2 and determined protein content of 58.6, 58, 57.7, 55.5 and 45.5 percent at different temperatures of 30, 35, 38, 40 and 42 °C, respectively.

Obgonda *et al*. (2006), the temperature of *Spirulina* sp. investigated its effect on biomass and protein biosynthesis. Protein content and amino acid compositions were measured at different temperatures, 25, 30, 35 and 40 ºC. They observed that the highest amounts of protein (46.39 g/100g) and amino acids (76.09 g/16 gN) were obtained at 30 ºC.

Koru and Cirik (2002) studied the biochemical composition of *Spirulina* biomass in outdoor cultures. Protein content was determined at different temperatures, 35, 37 and 42 ºC. They reported that when the temperature increased from 35 (64.7% protein) to 42 ºC (43.1% protein), significant changes occurred in the macromolecular composition of *Spirulina*.

Koru and Cirik (2003) conducted a study of *S. platensis* at 2000 lux in laboratory conditions. They investigated its effect on growth and metabolism at light intensity and temperatures from 28 ºC to 45 ºC. The effects of temperature increase on the morphology, growth, macromolecular structure, and fatty and fatty acid contents of filaments were tried to be determined. When the water temperature was 43 ºC, a significant decrease of 20% in protein content was determined. They determined that 35 ºC is the most suitable for biochemical structure and growth in intensive production of *S. platensis*.

Cyanobacterium *Spirulina platensis* has been studied by humans for its nutritional and possible medicinal effects. The study was carried out to evaluate the effect of temperature and nitrogen concentration in the environment on the biomass production of this cyanobacteria, and the change in protein, lipid and phenolic compounds. It has been reported that 35 °C has a negative effect on biomass production and a positive effect on protein, lipid and phenolic production, and the highest levels of these compounds are obtained in Zarrouk medium containing 1.875 or 2.500 gl^{-1} sodium nitrate. Higher biomass was

obtained at 30 °C than at 35 °C, but it was observed that nitrogen concentration had no effect on protein, lipid or phenolic content (Maria Colla *et al*., 2007).

4. PHYCOCYANIN CONTENT

Tomaselli et al. (1988) investigated how the filamentous structure, growth rate, macromolecular composition, lipid and fatty acid composition, and phycobiliprotein:chlorophyll-*a* ratio were affected by continuous production of *S. platensis* M2 at high temperatures and limited light conditions. In the same study, they examined the nutrient content of *Spirulina platensis* M2 strain at different temperatures and found 58.6% protein/dry weight at 30 °C, 58% at 35 °C, 57.7% at 38 °C, 55.5% at 40 °C and 45.5% at 42 °C. They stated that there was a 22% decrease in protein ratio in productions at 42 °C compared to those at 35 °C.

Among the proteins of *Spirulina*, the ones with the highest economic value are biliproteins. *Spirulina* contains two types of biliproteins, C-phycocyanin and allophycocyanin. About 20% of this microalgae protein fraction consists of phycocyanin, a water-soluble blue pigment. The maximum absorption of phycocyanin is 620 nm (Ciferri, 1983; Cohen, 1997).

In another study, in which we observed the effect of temperature on phycocyanin production, the effect of climatic changes such as seasonal temperature and light intensity on the amount of C-phycocyanin in *Spirulina platensis* was tried to be determined and carried out in Spring, Summer and Autumn seasons. C-phycocyanin and protein findings were recorded as follows depending on the temperature in the experiment (Table 4.)

Table 4. The effect of temperature on phycocyanin production in *Spirulina platensis*.

Spring	Summer	Autumn
Temperature (ºC mean): 24.87±0.74	34.52±0.60	30.03±0.43
C-phycocyanin (µg/ml, max.): 329.91±0.029	326.75±0.016	332.71±0.021
Protein (%) (End of growth): 68.15±0.52	68.16±2.13	72.09±1.04

(Oguz *et al.*, 2011).

5. *SPIRULINA* PRODUCTION IN THE COUNTRIES

In countries located at lower latitudes such as Thailand and Mexico, production facilities can produce for about 10-12 months, while those located at higher latitudes can produce for about 6-7 months. In California, *Spirulina* production can only continue for 7 months, from April to October, according to the maximum, minimum and average air temperatures recorded at Calipatria Weather Station from November to March.

One of the facilities where *Spirulina* culture is made in large volumes is Earthrise farm. Since 1990, Earthrise Farms has successfully overwintered *Spirulina* culture in open outdoor ponds, making the culture process faster and more economical. Although *Spirulina* has a long history of human use (Cifferi, 1983), commercial production of *Spirulina* in man-made ponds has been initiated by Dainippon Ink & Chemicals Inc. (DIC) was only pioneered in 1978 in Bangkok, Thailand. Earthrise Farms was founded in 1981 by the then US Proteus Corporation and later merged with DIC of Japan in 1982. Commercial production of *Spirulina* began in 1983 at Earthrise Farms in Calipatria, California.

Earthrise Farms was the first *Spirulina* farm established in the USA. With a total pool area of 75000 m^2 and an annual *Spirulina* production capacity exceeding 200000 kg, this is the world's largest food-grade *Spirulina* facility. Current (1993) and past producers of *Spirulina* in the World; In the international market, the annual production of Sosa Texcoco (Mexico), Siam Algae Company (Thailand), Cyanotech (USA), Earthrise Farms (USA) is 300000 kg, 75000 kg 110000 kg 143000 kg, respectively. domestic markets; Chile, India, Japan, P:R. China, Myanmar, Spain, Taiwan, Thailand. Failed projects include the French Indies, Israel, Japan, Spain, Taiwan and the USA (Belay, 1997).

FAO's data on *Spirulina* production in the world in 2019 are as follows; The total *Spirulina* production in the world is 56 208 tons, of which 54 650 tons were produced in China. This figure was followed by Chile with 861 tons, France with 201 tons, Greece with 142 tons, Tunisia with 140 tons, Burkina Faso with 140 tons, Central African Republic with 50 tons, Chad with 20 tons, Bulgaria with 2.65 tons and Spain with 1.30 tons. And at the same time, of the total production of 56 456 tons of microalgae in the world, 56 208 tons is

Spirulina. Fishery and Aquaculture Statistics. Global production by production source 1950–2019 (FAO, 2021).

6. CONCLUSION

Spirulina is a very resistant species against temperature changes, provided that it is not sudden. Therefore, its culture can be considered for many subtropical geographical regions and even *Spirulina* production can be carried out in many subtropical regions. However, can it be produced economically? You should think about it. Of course, the ambient and water temperature can be adjusted by consuming energy. Again, lighting can be provided by expending energy. Sometimes, investors insist that they can create optimum temperature and light and they want to realize their demands to produce *Spirulina*. In fact, the fattening medium is the main cost factor in *Spirulina* production. For these reasons, geographical regions with the most suitable climatic conditions should be preferred as much as possible for mass production of *Spirulina* and to make an economical production.

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CHAPTER 8

THE IMPORTANCE OF MICROALGAE: SOME CURRENT TECHNOLOGICAL TRENDS IN THE PRODUCTION AND BIOPROCESSING OF MICROALGAE BIOMASS

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1. INTRODUCTION

The world that is our home and everything. A place full of the unknown, but it is full of secrets where everything known about life begins. Life on Earth is a conundrum-all organisms need energy for living and function. But to harness that energy, the living rely on enzymes that have evolved over billions of years, to make possible everything from respiration to photosynthesis to DNA creat and repair. So under this circumstances what came firs, the enzyme or the organism? The latest research evidence suggests that the clusters of sulfur and iron at the heart of many life-critical enzymes could have been floating around Earth's primal seas some 4 billion years ago, produced by nothing more than primitive biomolecules, iron salts, and a formerly unknown component-ultraviolet (UV) light. Most research into life's origins has focused on how organic building blocks, like amino acids and nucleic acids, arose and assembled themselves into proteins and RNA. Generally revelant less studied is the genesis of iron-sulfur clusters, the active core in enzymes that drive almost every aspect of cellular chemistry. For all that research, genetic analysis studies suggests they've been around at least since the time of our last common ancestor. The first creatures of this environment were cyanobacteria and phytoplanton. Cyanobacteria played an important role in the evolution of Early Earth and the biosphere. They are liable for the oxygenation of the atmosphere and oceans since the Great Oxidation Event (GOE) around 2.5 Ga, debatably earlier. They are also major basic producers in past and present oceans, and the ancestors of the chloroplast. Even so, the identification of cyanobacteria in the early fossil record remains ambiguous because the morphological criteria commonly used are not always credible for microfossil interpretation. Recently, new biosignatures specific to cyanobacteria were suggested. Scientists still reviewing classic and new cyanobacterial biosignatures. The first Phytoplankton began to grow in abundance in the oceans about 3 billion years ago and have since replaced carbon dioxide, the main component of the atmosphere, with oxygen through their photosynthetic abilities, creating the current atmospheric composition.

The oxygen produced by photosynthesis not only created the atmosphere, but also raised the dissolved oxygen level in the seawater. As a result, the iron in seawater was oxidized and deposited on the seafloor, eventually forming the current iron ore layer. Large quantities of dead

microalgae were also deposited on the seabed and, billions years later, they became oilfields (Figure 1).

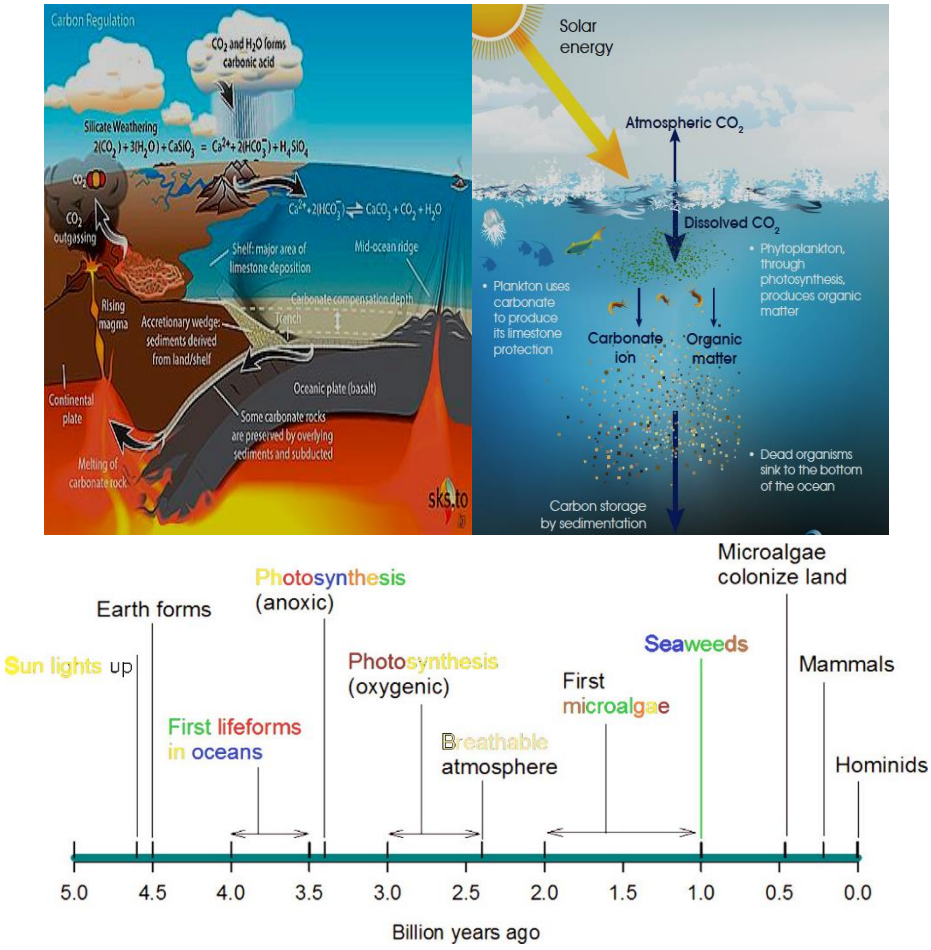

Figure 1. Geochemical and oxygen cycle in the world (Chisti, 2020; Wikipedia, 2022;Shapeoflife, 2022)

Gephyrocapsa sp. (Fig. 2), a type of Haptophytes, causes carbon dioxide to react with calcium in the water, producing calcium carbonate, thus forming the circular outer shell of the cell (Guiry&Guiry, 2008; Bendif and Young, 2014). As aresult of reaction has limestone layers formed by the process.

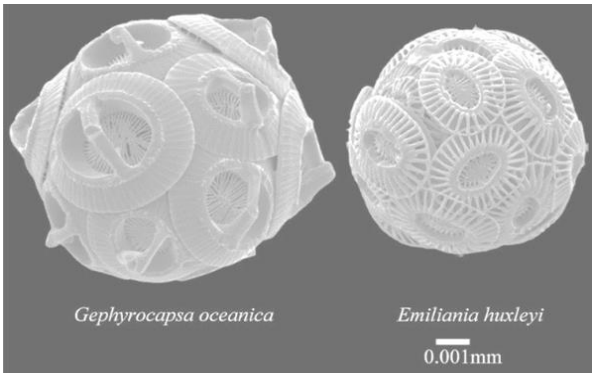

Figure 2. Some *Gephyrocapsa* species (Bendif and Young, 2014)

Microalgae make up the lower part of the food chain - they are eaten by zooplankton (grazing) which is eaten by small fish, then by large fish, and then by humans, also eaten by farm animals. In this way, microalgae make up the current global environment and nourish living organisms on Earth even now. Humans exploit and benefit from the resources produced by microalgae (Sumi, 2009). And also, they are evaluating assess the reliability of the previously described cyanobacteria fossil record and the challenges of molecular approaches on modern cyanobacteria. Despite all the uncertainties, many scientists believe that RNA, or something similar to RNA, was the first molecule on Earth to initiate the process of self-replication and evolution that gave rise to more advanced life forms, including humans (Schirrmeister et.al., 2013; Klatt, 2021). As a result, it is a fact that the biggest source of oxygen production in our world is cyanobacteria, micro-macro algae and other plant-based creatures. Microalgae, which have such an important effect on the atmosphere and life in our world, also have valuable biochemical properties. From the Great Oxidation Event (GOE) 3 billion years ago to the present, cyanobacteria and microalgae are the most important creatures in terms of oxygen potential to support life (Fig. 1). And also, microalgae or plankton are natural biological factories that are rapidly getting topical prominence due to their longterm sustainable and all-purpose applications as food, chemistry, cosmetics, medical applications and feed industry for biochemical and bioenergy production and in the reduce of global climate change like carbon dioxide emissions (Selvarajan et al., 2015; Liu et.al., 2016; Zhang et.al., 2019;). Biomass of microalgae is a natural food source for many considerable

aquaculture organisms such as mollusc, shrimps, fish and feed industry (Mutanda et al., 2020).

The microalgal biotechnology industry has received significant attention in recent years owing to biorefinery whereby a varied of products e.g., carbohydrates, lipids, proteins, nucleic acids, chlorophylls, carotenoids and nutraceuticals, are generated from the identic harvested microalgal biomass within a same time (Camacho et.al., 2019; Kumar, et.al., 2020). The commercial cultivation of microalgae in the world was for the first time in aquaculture depending on the nutritional requirements of the larval stage. In aquaculture, they are used as live food for all growth stages of bivalve mollusks (e.g. oysters, scallops, mussels and mussels), for juvenile stages of abalone, crustaceans and some fish species, and for zooplankton used in aquaculture food chains (Sirakov et.al., 2015).

2. WHAT ARE MICROALGAE ORGANISMS? WHY THEY ARE IMPORTANT IN THE ENVIRONMENT AND INDUSTRY?

The algae, especially microalgae, are the world's oldest living organisms that form the basis of the aquatic food chain. They are also called phytoplankton. Microalgae are phylogenetically diverse groups consisting of many different phyla and classes of organisms, including in some cases cyanobacteria (Kay and Barton, 1991; Hayes et al., 2018). in soils and rocks (Dittami et al., 2017; Camacho et al., 2019). Microalgae are ubiquitous eukaryotic photosynthetic microorganisms that are found in diverse normal and extreme aquatic habitats such as freshwater, lacustrine, lake (i.e. fresh-brackish-soda-brine water), river, marine, estuarine, brackish, thermophilic, saline and hyper-saline environments (Meeting, 1996; Leliaert et al., 2012). Hence, microalgae can be found in almost all ecosystems (Selvarajan et al., 2015). However, cyanobacteria are blue-green photosynthetic prokaryotic microorganisms that fall within this broad limitation (Lee et al., 2014). In this vast ecosystem, phytoplankton are widely important primary producers and form the basis of the food chain in aquatic environments (Malapascua et al., 2014). With an estimated 200,000 to several million different strains, microalgae have enormous biodiversity compared to terrestrial higher plants with only about 250,000 members (Delrue et al., 2016). Microalgae are diverse

phytoplanktonik organisms that produce a variety of metabolites under photoautotrophic, heterotrophic and mixotrophic growth conditions. Local marine, freshwater and brackish water microalgae are an extremely heterogeneous group of oligotrophic organisms with minimum nutrient requirements hence they can thrive in any natural environment (Vu et al.,2018). Their sizes can range from a few micrometers (μm) to several hundreds of micrometers that depending on the species (Suganya et al., 2016).

The growth kinetics of microalgae change due to effective key growth controlling factors. Microalgae are attractive candidates for high-value metabolite generation due to their fast growth rates as compared to some terrestrial plants (Kirrolia et al., 2013). Phytoplankton, also known as microalgae, are similar to terrestrial plants in that they contain chlorophyll and require sunlight in order to live and grow They have as a new material in biotechnology production systems are positioned at the connection of these diffuculties as many species have high efficiencies relative to conventional yield in terms of using solar energy to drive the conversion of CO_2 to biomass (stored chemical energy). This biomass can subsequently be used to produce a broad range of downstream products. It has been widely stated that microalgae have the advantage that they can be produced on a proportion of non-arable land (nonarable land is ~25% of global surface area vs. ~3% arable land area and in many cases can use saline and waste water streams. This theoretically may be opens up the opportunity to extend global photosynthetic capacity beyond arable lands and support with a transition from the available food and fuel position to a more sustainable ″food and fuel″ future. However the simplicity of the concept has not progressed to commercial reality despite a significant international research effort. This is primarily due to the many interconnected challenges of optimising biology and engineering parameters for high efficiency production and integrating these into commercially viable systems.

Newly emerging strategies for high efficiency microalgae production (Fabris et.al., 2020) may contribute significantly to a food and fuel future but they are not the panacea that some have promoted. Dissident opinions that microalgal production systems lack the appropriate production strains suitable to overcome the challenges of economic and environmental sustainability for competitively priced biofuel production may be valid at the present time. But

such arguments are insubstantial given the early stage of technology maturity, the rapid ongoing development in the field currently, and the large microalgae biodiversity (~300,000 species) and advanced genetic engineering techniques that can be stepwise for strain optimisation (Meeting, 1996). Utilizing microalgae as a biological resource is clearly an advantage and presents a considerable undertaking. Phycologists have been isolate until now ~10,000 microalgal strains until today (Susanti and Taufikurahman, 2020). That's why still, there is a huge potential for this untapped and unexplored broad bioresource for potential biotechnological applications (Delrue et al., 2016). However, high-throughput processes for strain isolation and maintenance are absolutely necessary to improve the efficiency of traditionally laborious methods in algae production.

3. APPROACH TO THE CONCEPT OF BIOREFINERY IN MICROALGAE PRODUCTION

The world population is expected to increase from its current level of ~7 billion to ~9 billion by 2050. This progress is coupled with an extraordinary level of lifestyle change in developing countries. It is estimated to result in ~70% more food and ~50% more fuel by 2050, as well as fifty percent more fresh water and increased chemical feedstock requirements (Haupt, 2011; Anonymous, 2022). A shift from fossil fuels to renewable systems is needed to reduce global CO_2 emissions. provide these resources. The scale of this challenge should not be underestimated given the urgent need to significantly reduce CO_2 emissions this decade if we are to stay within the 'safe margin' (2°C) defined by the Intergovernmental Commission on Climate Change. This is a promising target, given recent claims that 80% of the remaining fossil fuels must be left in the ground to prevent progress beyond this threshold. Unfortunately, the Covid 19 pandemic due to the balance of all systems in the world has been disrupted and brand new practices and policies have emerged for the continuity of life.

The term “microalgae” is generally used for both prokaryotic blue green algae (cyanobacteria) and eukaryotic microalgae including green algae, red algae, and diatoms. Microalgae are being sought as alluring biofactories for the sequestration of CO_2 and simultaneous production of renewable biofuels, food, animal and aquaculture feed products and other value-added products such as

cosmetics, nutraceuticals, pharmaceuticals, bio-fertilizers, bioactive substances (Singh and Dhar, 2019). Microalgae are a up-and-coming alternative to tradition crops in terms of craft the world's growing food and energy needs without negative environmental impacts (Fig. 3).

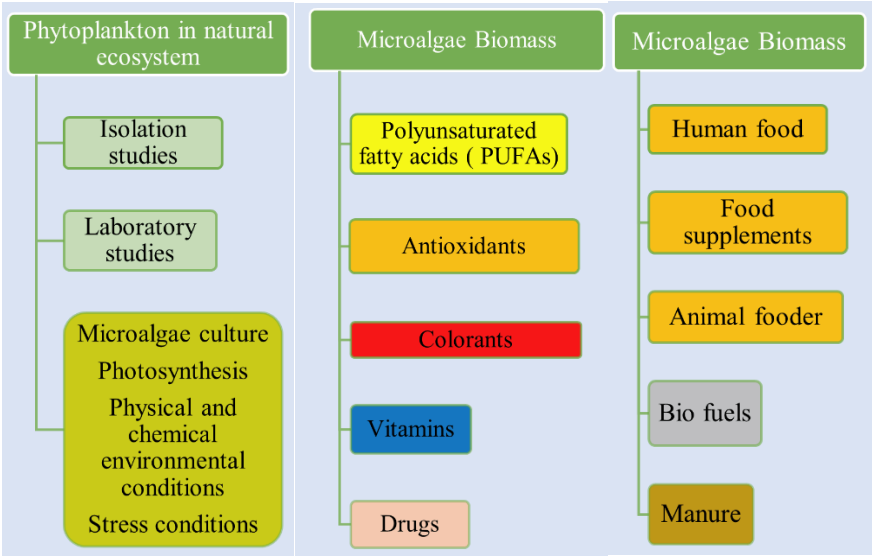

Figure 3. Common uses of microalgae.

The biorefinery definition is "the sustainable processing of biomass into the spectrum of marketable bio-based products (chemicals, materials) and bioenergy (fuels, power, heat)", according to the International Energy Agency (Fig. 4-5). For this reason, biorefineries that can evaluate all components of microalgal biomass with appropriate side-treatments have the greatest chance of success. The most important factor here is the energy and raw material costs used in the production process.

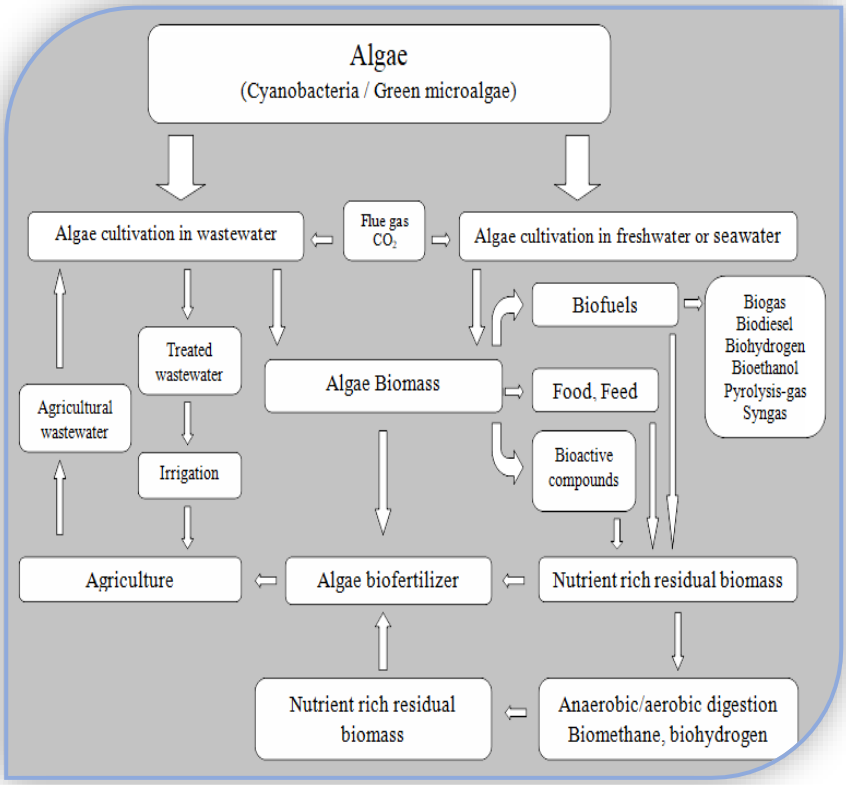

Figure 4. Generally shematic diagram of a comprehensive biofenery approach to microalgal biomass processing (Debowski et.al., 2020)

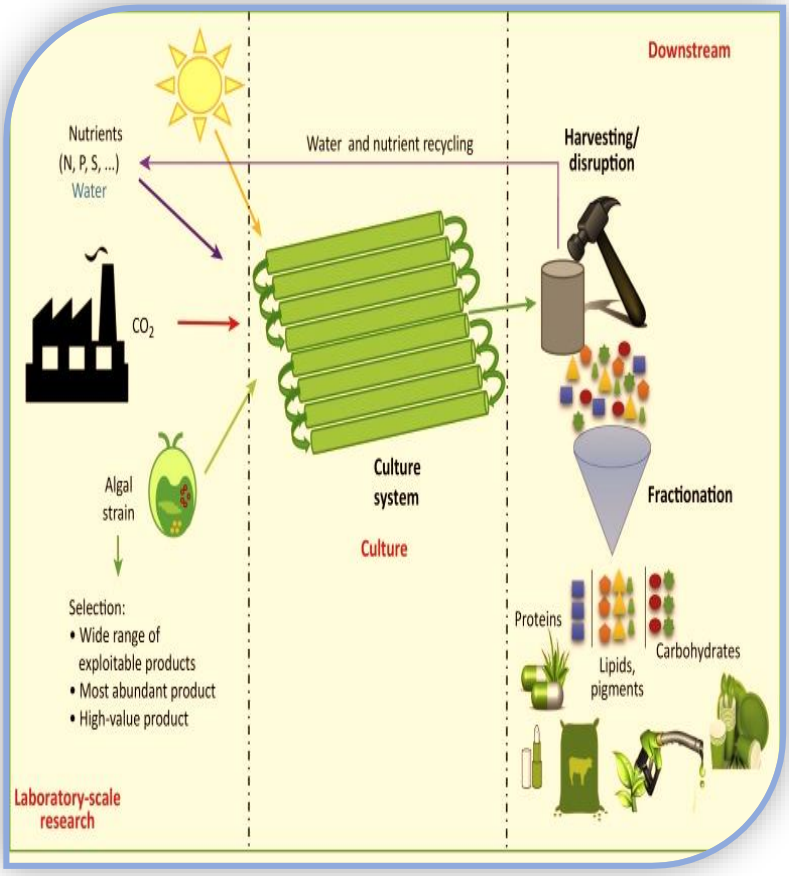

Figure 5. Common Microalgal Biorefinery Integrating Separate Operation Units: Laboratory-Scale Research, Culture, and Downstream Processing. Laboratory-scale research includes selecting a microalgal strain based on the main target product required and the sources and the supply of low-cost nutrients for microalgal growth. The culture step concerns the construction, operation, and optimization of a culture system that (i) better fits the microalgal strain requirements in terms of mixing strategy and light penetration, (ii) reduces the investment and operational expenses, and (iii) provides sufficient algae biomass concentration and productivity for efficient downstream processing. The downstream unit is composed of (i) harvesting for biomass concentration- mechanical seperation, (ii) extraction of intracellular molecules by cell disruption or chemical extraction, and (iii) fractionation of the molecules to maximize the number of commercial products obtained (Gifuni et. al., 2019)

Despite of the great expectancy shown by the algal biorefinery, the current limitations need to be addressed. The total quantity of microalgal biomass produced in current industrial processes is disappointingly low (around fifteen to twenty thousand tons per year) and production processes are usually not economically viable (Hariskos and Posken, 2014). High-value products from microalgae are usually significantly more expensive than their chemically produced counterpart and serve niche bio-markets. When they have photosynthesizing, phytoplankters convert atmospheric CO_2 into carbohydrates, lipids and other valuable biological components using light. Therefore, microalgae biomass is a rich source for food and bioactive compounds, as well as for biofuel production. So, microalgae biomass is a rich source for food, biofuels and bioactive compounds

4. MAINE FACTORS ON MICROALGAE CULTURING

Microalgae can be cultured by different methods and under different conditions in production systems or biorefinery. Phytoplankton need light as an energy source like sun to convert the absorbed water and CO_2 into biomass through photosynthesis. Photosynthetic products accumulate in various forms, such as cell components or storage materials, and vary from 20 to 50% of total biomass source to convert the absorbed water and CO_2 into biomass through photosynthesis (Fig. 6).

When culture conditions are suitable for microalgae are rapidly growing photosynthetic organisms having potential of transforming 9-10% of solar energy (average sunlight irradiance) into biomass with a theoretical yield of about 77 gr./biomass/m^2/day which is about 280 approximately ton/ha/year (Melis, 2009; Formighieri et.al., 2012). Therefore, large-scale (from the test tube to the production pond) microalgae cultivation will decisively contribute to the development of a sustainable industry for biomass production as well as creating cost-efective high-value products. Many species of microalgae show potential for large-scale cultivation, notwithstanding there is insuffcient information to run commercial trials. A huge amount of microalgae biomass is required to compete with other feedstocks for sustainable production for commercial addet value. Successful microalgae culturing technologies will need to create larger amounts of biomass, which will make the use of food stufs for valuable production comparatively less attractive (Khan et.al., 2018). The

various metabolic compounds in microalgae will become even more important and valuable commercially.

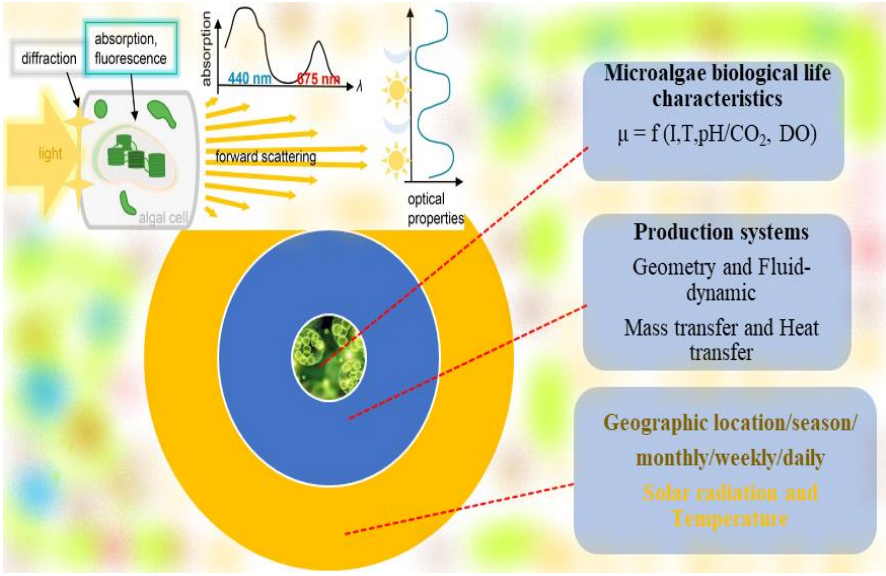

Figure 6. Fundamental parameters determining the productivity of microalgae-related systems (Lehmuskero et. al. 2018)

5. MICROALGAE PRODUCTION SYSTEMS

Microalgae are classified into four types according to their reproductive characteristics: photoautotrophs, heterotrophs, mixotrophs and photoheterotrophs (Fernandez et.al., 2020). In the entire microalgae production sector, microalgae is shaped according to this main biological feature. Many production systems are available to obtain microalgae and its biomass. These systems differ according to microalgae biology, production conditions, product quality, geographical location, climate, cost and intended use of the product. We can summarize these systems as follows: Closed production systems and open production systems. Closed systems photobioreactors and fermenters in production (Fig.7). Open production methods are bag, light paths and pool systems (Fig. 9).

Whatever the application in closed systems, the core of the process is the photobioreactor on which the process is performed, which must be designed according to (i) the microorganism to be produced, (ii) the quality of microalgae

biomass required, and (iii) the whole production capacity. In this meaning, largely various photobioreactors are used at commercial scale, from polythene little bags used in aquaculture to produce small amounts of high-quality biomass of phytoplankton strains, to tubular photobioreactors developed for the production of tons of high-value and sensitive strains such as *Chlorella* sp., *Haematococcus* sp., *Scenedesmus* sp. and *Nannochloropsis* sp., or large open raceways used for the production of hundreds of tons of extremophile strains such as *Arthrospira* sp., or *Dunaliella* sp. (Fig. 8).

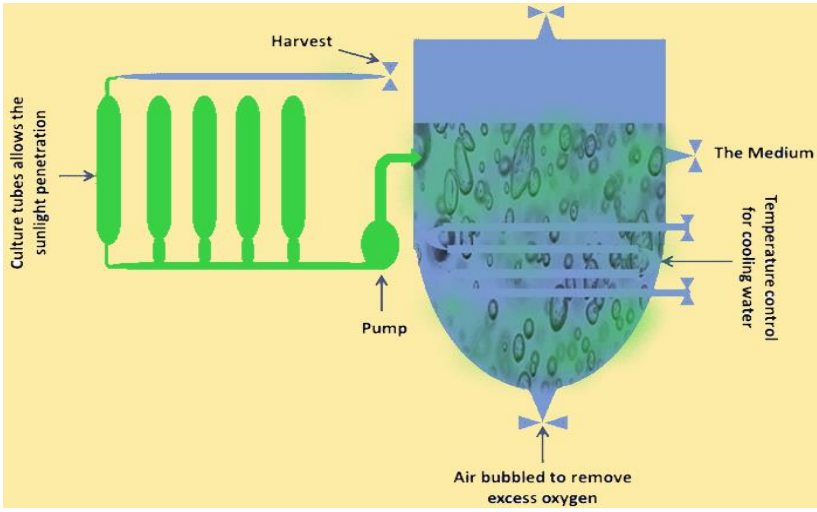

Figure 7. A general bioreactor's working principle

Due to photosynthesis reactions, light is the main physical factor in conventional production systems. Microalgae can also use organic compounds as an energy source, then grow in heterotrophic mode or can also grow in myxotropic growth if light is also provided. In this case, it is recommended to use traditional fermenters. It is also possible to use artificial lighting instead of natural sunlight for autotrophic microalgae production. However, due to the low light conversion efficiency of photosynthesis, the production process (>10%) is only suitable for very specific conditions such as non-energy supply or very high value biomass production.

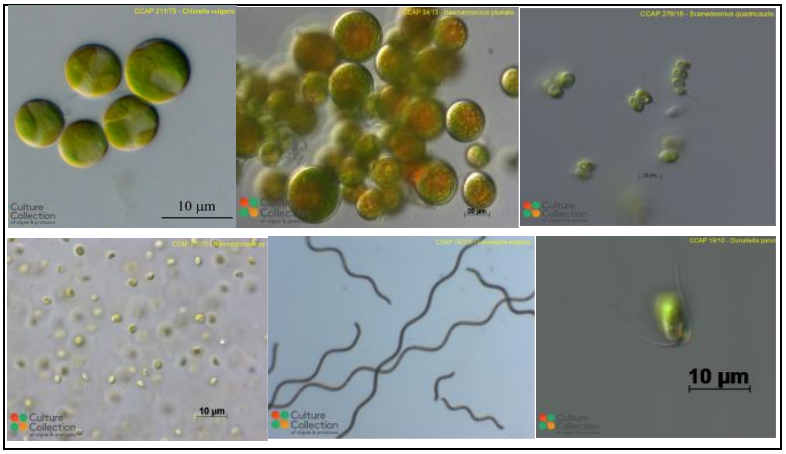

Figure 8. Some phytoplanktonic organisms used in microalgae production (https://www.ccap.ac.uk/).

Raceway Pons

Raceway ponds are currently the most preferred reactors for the production of microalgae worldwide; more than 90% of worldwide production is performed using these reactors, due mainly to its low cost (Fig. 9).

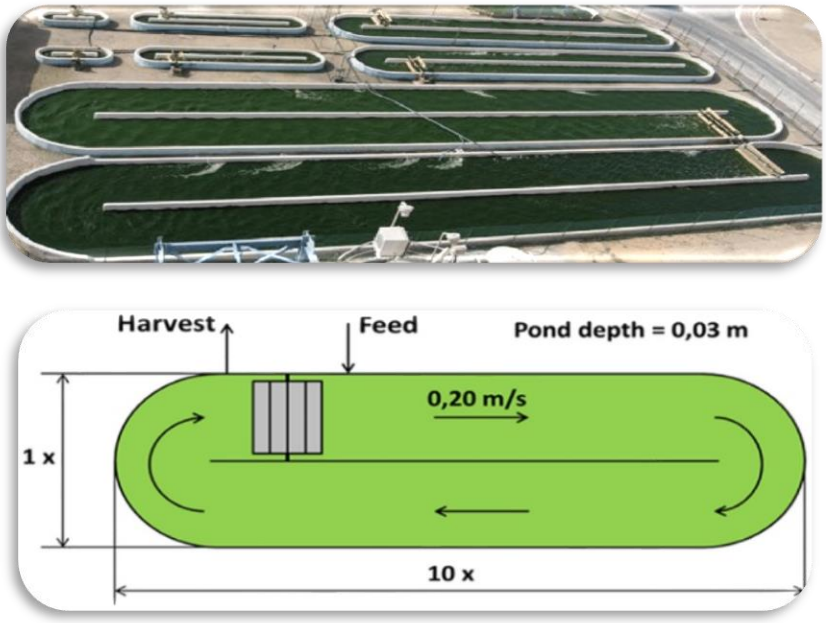

Figure 9. Raceway production system (Kumar & Jan, 2014)

The cost investment for these systems ranges from 0.13 to 0.37 M€/ha at 100 ha scale (Norsker et al., 2011; Chisti, 2012). Plant producing microalgae in raceway reactors are located worldwide, such as the United States,China, Thailand, Chile, France, Spain, Italy and other countries; the biomass produced is mainly used for human consumption. In raceway ponds the biomass concentration remains below to 1.0 g/L due to the high water depth; this facilitates the contamination of the cultures by other microorganisms and increases the risk of failures, both very reducing the overall productivity of these systems. In order to minimize these problems, only robust and fast-growing strains, tolerant to extreme conditions, are produced in these reactors, such as *Spirulina* sp., *Chlorella* sp., and *Dunaliella* sp., Although biomass productivities up to 40 gm^2/day (equivalent to 150 t/ha/year) have been reported (Lundquist et al.,2010), on average, much lower productivities are usually claimed, in the range of 9-15 g/m^2/day when producing *Tetraselmis suecica* or *Nannochloropsis* sp. (Chiaramonti et al., 2013), 13 g/m^2/day when producing *Chlorella* sp., or 21 g/m^2/day when producing *Spirulina* sp. (Richmond, 2004). The credibility of raceway reactors also depends on additional factors such as climatic conditions, the presence of dust and pollutants, the presence of biological contaminants such as insects or fungi, bacteria, etc. Regarding the design in channel pools, the overall design of any channel reactor usually consists of two channels through the culture circulated by a paddle wheel. This provides energy to overcome the pressure drop in the system, especially on bends. Channel pools are built on compacted soil coated with polymers, but it is also recommended to use only compacted soil for cost advantage. Even thought some designs including up to four channels have been proposed, they are not recommended due to the larger pressure drop caused by the additional bends. The length-to-width ratio of this type of reactor ranges from 10 to 20, and the utilization of lower length/wide ratios is preferable to reduce the pressure drop into the system, especially at large scale. Due to the limited capacity of the paddlewheel to provide energy, raceway reactors up to $5m^2$ are constructed at industrial scale; larger reactors are not generally feasible because of the large pressure drop into the channels and bends. Commercial facilities are then composed of multiple units of this industrial size standard unit. The water depth into the raceway ranges from 0.2 to 0.4 m, low water depth being recommendable to increase the light availability and then the biomass

concentration inside the culture (Fig. 9). Opposite, high water depth is more recommendable when using the raceway reactor to treat wastewater because of the larger volume of the reactor and then the larger wastewater treatment capacity for the same hydraulic residence time.

6. CONCLUSION

High biomass amounts of compounds are needed in various production areas to enable successful production of microalgae-derived products. Different conditions and methods in commercial production models allow (1) suitable light sources, (2) efficient working procedures, (3) minimization of contamination rate, and (4) reduction of production costs (5) optimal and inexpensive harvesting of biomass. As in the microalgae evaluation process, the effect of the methods also affects the production purpose. Large-scale cultivation of microalgae is usually done in open ponds and various types of photobioreactors. However, there is a possibility of contamination and contamination in open pools and uncontrolled optimal conditions. This model of production is the type of growth that makes this possible only under certain microalgae species and conditions. Microalgae biomass has become an inspirational resource energy with versatile applications in the production of sustainable foods, biofuels and dietary supplements. Therefore, mass cultivation of these natural biofactories has been contemplated over the past few 20 years. Depending on the developments in technology, methods for biomass production and evaluation have been further developed and biofenery systems have been created. Microalgae cultivation has some advantages in the traditional farming process. These are high productivity, high lipid content and other bioactive components, the ability to grow in wastewater, and the need for large farms are the advantages of algal biomass production compared to conventional crops. In the past, the use of microalgae as a renewable energy source (biofuels) and environmental applications, large-scale industrial production microalgal biomass has been proposed. However, this has not been a commercially sustainable production model. So nowadays, it is possible to use algae for human consumption as the most suitable production purpose as a traditional small-scale agricultural activity. Therefore, the first sure step in the commercialization of microalgae production is the selection of suitable species and strains for cultivation. Selected strains must be able to withstand a wide

range of environmental light, temperature, salinity, growth medium and pH conditions.

The important research and development target in this field is to develop "domesticated" strains of microalgae with a high feed or fuel value that can dominate in the large open, unlined, raceway ponds, with their complex fluctuations in light, temperature, nutrients salt, O_2, and pH. Dominance means stable cultivation, meaning resistance to invasion by microalgae, grazers, paras itic fungi, viruses, bacteria etc. The microalgal strains thus developed must also be able to be cheaply harvested and processed. A major R&D effort will be required to develop such strains, tolerant of the pond environment, resistant to biotic challenges, versatile in outputs, and more highly productive than any current agronomic system. There is reason for optimism that it may be possible to achieve these goals. Developments in biotechnology also make significant contributions to this issue. As noted earlier, several microalgae species are already commercially produced in canal ponds, albeit inexpensively or on a large scale, or at high productivity, although not in large numbers required for fuels and feed. However, some additional species have also been bred on a relatively large scale. Thanks to the advancement in technology, it is possible to produce strains with less cost and product variety. Appropriate, strain improvements are essential requirements for microalgal feeds or fuel production.

As a result, the algal researches and developments target in this field is to develop "domesticated" strains of microalgae with a high feed or fuel value that can dominate in the large open, unlined, raceway ponds, with their complex fluctuations in light, O_2, pH and temperature. Dominance means stable cultivation, meaning resistance to invasion by microalgae, grazers, paras itic fungi, viruses, bacteria etc. The microalgal strains thus developed must also be able to be cheaply harvested and processed. A major R&D effort will be required to develop such strains, tolerant of the pond environment, resistant to biotic challenges, versatile in outputs, and more highly productive than any current agronomic system. There is reason for optimism that it may be possible to achieve these goals. Developments in biotechnology also make significant contributions to this issue. As noted earlier, several microalgae species are already commercially produced in canal ponds, albeit inexpensively or on a large scale, or at high productivity, although not in large numbers required for

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CHAPTER 9

STATISTICAL DATA COLLECTION IN AQUACULTURE

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1. INTRODUCTION

The main aim of science is to understand and explain natural and social phenomena based on the results of experiments and extensive observations. Observation and experimentation are the two main ways of producing "knowledge" about the world. The scientific method, in addition to observation and experiment, also includes description and theoretical explanation. For this purpose, standard methods or procedures have been developed and used to conduct scientific research or research. The scientific method consists of collecting, compiling, analyzing and interpreting data from reliable sources or well-designed experiments. Scientific research is a long process and hard work.

A researcher's knowledge and skills in conducting research are reflected in their publications and presentations of research findings. In this book chapter, how to collect data to be used in aquaculture research and because there is a need for a better understanding of statistical science, statistics have become a necessity for researchers to assist researchers who encounter problems.

Those with statistical knowledge use at least percentage or probability. Statistical skill and knowledge therefore give individuals the ability or power to interpret and draw conclusions. It also teaches techniques for presenting research results accurately and assists in the critical evaluation of published or planned literature. Considering the various uses and importance of statistics, the science of decision making under uncertainty has been defined as a set of methods and theories applied to numerical evidence in decision making in the face of uncertainty, a toolkit for problem solving (Sokal and Rohlf, 1995).

Consequently, statistics should be considered not only as a branch of mathematics, but also as a fundamental background for researchers, which eventually becomes a way of life. More importantly, it is a logical way of thinking that is necessary for everyone.

Attempts have been made to define statistics based on practice. For the biological sciences, statistics is the measurement of living organisms. Biological phenomena are very diverse and influenced by many causal or environmental factors, and the factors themselves are variable, uncontrollable, and often unidentifiable; therefore, a fishpond is considered a black box. They are probabilistic in nature, or statistical thinking, meaning there is nothing that is absolutely true and absolutely false. It is also referred to as "Biostatistics",

which means the application of statistical methods to the solution of biological problems. The use of statistics has become necessary in almost every field**.**

2. AQUACULTURE

Although fish farming dates back about 4,000 years, FAO data show that the real growth of this area only began after the 1980s. It has now become the fastest growing food production sector. The only way to compensate for the decline in hunting production is to increase aquaculture production. It is expected to grow even faster to meet production demand of 80 million tons (almost twice the current level) by 2050, according to FAO's forecast. However, at the moment there are numerous difficulties in this regard. There is an urgent need for further research in various disciplines for the further development of this field, such as: Increasing environmental problems caused by aquaculture, Introducing new aquaculture species that pose a threat to native species, Improvement of native species breeding, maintenance and breeding techniques, Increasing disease problems as a result of cross-border movement of aquatic species and intensification of aquaculture systems, Development of low-cost feeds from locally available raw materials, Substitution of fish meal and fish oils used in animal feed production with other raw materials, Economic studies or comparative studies with other sectors for the feasibility of aquaculture, Little is known about the roles and interactions of micronutrients, eg. minerals, vitamins and fatty acids, More work on technology transfer or adaptive research and participatory farm trials, Food safety and quality

There is so much research to be done for the full development of the aquaculture sector; however, most aquaculture scientists/researchers lack statistical knowledge and skills. Statistical infrastructure is required so that researchers can design appropriate scientific experiments, analyze and interpret data accurately, and present them appropriately. Many aquaculture researchers have to seek help in designing experiments and analyzing and interpreting data. They also face great difficulties in publishing research papers. As a result, many do not share results after doing research, even if they have very fruitful findings; Therefore, the entire aquaculture industry is in distress.

3. EXPERIMENTAL UNITS IN AQUACULTURE

A clear understanding of the experimental units and system is essential when planning and designing research. Various factors need to be considered, including costs involved, objectives or targets, facility availability, feasibility, management, climatic conditions, and trial duration. In this section, experimental systems commonly used in fisheries research;

Earthen ponds: The size of the ponds used varies depending on the farming purpose and the availability of the land. A fry pond can range from 50 to 200 m2, while a breeding pond can range from 500 m2 to 1 ha or larger. However, ponds as exploratory experimental units should not be large, as the land itself is expensive and surveys requiring adequate replication are costly. Normally 50–100 m2 should be sufficient for brood rearing and 200–400 m2 for breeding trials. The environmental conditions in a pond will be uniform if all ponds are supplied with the same water, as nutrients or chemicals can freely disperse in the water, unlike a piece of land where the fertility of the soil varies greatly. However, some environmental parameters that are directly or indirectly related to the survival, growth and reproduction performance of fish in ponds may differ. For example; Birds can be a problem because they can eat the fish used in the experiment. Predatory fish and other animals can enter the ponds. During the rainy season, especially in low areas, flooding can be a problem and all experimental fish can be caught in flood waters.

Ponds and cages: Research using ponds reused is extremely difficult unless it is a large facility or there are smaller specially designed ponds available. In some cases, this can be extremely expensive. However, a pool or cages can be set up to divide it into several experimental units, or the pools can be set up in rows. There may be variability from the edge of the pool to the centre, as there is a greater probability of noise and shallowness at the edge than at the center of the pool. If ponds or cages are arranged in rows, the rows should be considered as blocks so that variability can be separated when performing statistical analysis. As there is no free movement of nutrients/chemicals from one pool to another, there is a high probability that one pool will be different from the other. Therefore, if multiple pools are used for the same trial, each pool should be considered a block, meaning that each refinement must be

randomly distributed across each pool. However, in such a situation, the pools or blocks again act. Ponds or cages have the advantage for experimental purposes over ponds as birds can be avoided as they are covered. Also, cages and ponds may rise if the water level rises to prevent fish loss due to flooding. However, cages and ponds can be expensive and take time to set up. It is easy to steal fish from ponds or cages, although fish are easier to sample and collect.

Cages in lakes or reservoirs: In many places, lakes or reservoirs can serve as experimental facilities. Due to their large size, several test units can be set up in the same water; therefore, this is suitable for a completely random design. There is a low probability of variability or a higher chance for any application to show its true effects. However, care should be taken if cages are present on or near lakes or reservoirs where sunlight or shade may affect rows on or near the shore more than others. In this case, the lattices can be arranged in rows along the edge, the rows can be considered as blocks. A random full block design would be appropriate.

Tanks: Researching in tanks gives more control over environmental factors and is also easier to research than in caged, mesh, or small-mesh ponds. Various types of tanks can be built or selected, eg. circular, square or rectangular. However, building a tank can be costly and care must be taken when designing a trial; A small mistake can ruin research and requires repetition. If the tanks are supplied with the same source of water circulation (bio- or mechanically filtered), the variability in water quality parameters other than temperature will be very low. On the other hand, external tanks without water circulation/exchange vary greatly. Tanks close to walls and water supply channels can affect water quality parameters and therefore fish growth and survival. Therefore, it is necessary to monitor or measure the water quality parameters of each tank. Because the tanks are smaller in size, the productivity or efficiency of the system is normally expressed or compared in kg·m-2 or kg·m-3.

Aquariums: Aquariums are the best experimental units for small scale experiments, especially in a laboratory or shade fish hatchery. They are easy to install and handle. Researchers may in most cases have multiple experimental

units; therefore, they will not be constrained by plant, lack of repetitions or treatment levels. They can even be stacked on top of each other. Height can be an obstacle in such a case. A researcher can gain better control over environmental factors, which means there is less chance of masking the real effects of practices. Most breeding and some nutritional research is done using an aquarium. However, the results of trials in the aquarium may have limited application on a commercial scale. Arrangement of experimental cages in the lake. Cages in a lake designed for a research station.

Research with the participation of farms: Any research conducted at research stations or university facilities is called station research, which is directly controlled by people who are well acquainted with the research goals or objectives. As the ultimate purpose of research at the station is to serve a larger population, research results will need to be tested in the field situation where there is less control over factors. Before making any policy proposals, they should be piloted and determined whether they work for real beneficiaries. On the other hand, government extension organizations can conduct pilot studies using the facilities of selected farmers who can follow a set of guidelines. Research trials conducted using the facilities of the final beneficiaries of the farms are called participatory research. At the same time, most extension workers feel that their responsibility is simply to pass on research results and well-developed technology packages to farmers. However, since no technology is perfect, they must be constantly improved and adapted to specific local conditions. While the control of factors is limited, research results can be extremely valuable because they are drawn directly from exactly the same conditions and are therefore directly applicable. Participatory research is difficult to manage because suitable farms may be far apart, and the researcher also has to deal with many people from a wide variety of cultural backgrounds, beliefs, education and/or income. Sometimes farms' facilities, even for free use, can be very costly, as they may need to be adjusted or upgraded to meet research needs.

As a result, the points to be considered in determining the study area can be summarized as follows. A researcher should be very clear about which experimental unit to use. Individual fish in the same aquarium, tank, cage, net, or pond can be test units or replicates if all fish are labeled.

The choice of breeding system for research is up to the researcher. Unless there is a special need, aquarium or tanks should not be chosen simply because they are easier and cheaper to manage. The result of the research may have limited application in the real field situation. On the other hand, research in larger experimental units is unnecessary if they cannot be managed. Sometimes, failure to properly control or monitor potential factors turns out to be a waste of time, resources, and effort. Using relatively smaller experimental units and a well-planned and properly managed experiment can generate sufficient data. Appropriate statistical analysis can provide enough information to make precise and useful inferences.

4. SAMPLING AND DATA COLLECTION

All observations of all possible organisms, objects, or a particular feature of interest in a given geographic area are populations. Measurement of whole organisms may be possible in some research trials, but most of the time monitoring the entire population is difficult, costly, impractical and often impossible. For example, it is possible to individually measure up to 50 or 100 fish in several test tanks or aquariums. But if tanks are larger, with 200 or more fish in each, taking individual length and weight measurements is tedious. Also, if we need to find the average weight of a fish species in a reservoir or lake, it is not possible to catch all fish of that species. Therefore, instead of monitoring or measuring whole organisms, manageable portions of the population are taken as samples. There is no hard and fast rule about sample size. It is very diverse, eg. 1%, 5%, 10%, 25%, 50% and even more depending on the situation. The larger the sample, the higher the accuracy and reliability, but the cost increases with sample size. Therefore, there is a trade-off between cost and accuracy. The key point here is that the sample should be minimal, but that all members of the population have an equal chance of being selected and that the sample should be taken randomly so that it is representative of the entire population. Appropriate sampling techniques or methods should be followed to ensure that the samples are representative. The main sampling methods are:

Random sampling: Common method in which samples are randomly selected from a population in a single step. For example, randomly selecting 100 fish without any bias for size, color or other characteristics from a tank

containing 500 fish to measure individual weight and length. The entire experimental system needs to be randomly sampled from each replicate, group, or stratum. Random sampling: Common method in which samples are randomly selected from a population in a single step. For example, randomly selecting 100 fish without any bias for size, color or other characteristics from a tank containing 500 fish to measure individual weight and length. The entire experimental system needs to be randomly sampled from each replicate, group, or stratum.

Systematic sampling: sampling performed at specific time intervals. For example, monitoring pH, water temperature, and dissolved oxygen each morning, noon, and evening as the operation continues, when they are at their minimum and maximum, respectively.

Stratified sampling: a representative method of sampling from all strata or groups. For example, groups of large, medium and small sized fish to represent the entire population of different sizes. The factor group can be included as a block when analyzing the data.

Cluster sampling: It involves selecting specific groups or clusters first to avoid species that do not need to be included. Then random selection is made within each cluster. For example, choosing 10 out of 81 cities representing Turkey to examine aquaculture development, then randomly selecting 40 aquaculture businesses from each city for interview.

Multistage sampling: when the population is very large, sampling should be done several times rather than once. For example, it shows that the initial sampling is random. In the second step, samples can be taken from two opposite sides (up and down) from three quarters of a circle. Then in the third step, again choosing the opposite sides (left and right) can produce a good example.

5. PARAMETERS or VARIABLES

The properties of the populations are called parameters. An example is the population or actual average of 30,000 fish grown in a 1 hectare pond, which

is a parameter and is almost impossible to find by measuring one by one. 00 Parameters must be estimated by sampling and may vary with sampling method and other factors. The word "variable" is widely used; reflects characteristics in which individual organisms or objects vary in a determinable way due to some cause(s). Causes are factors and are often called independent variables, and effects are evaluated in terms of characteristics.

5.1. Variable types

Researchers should be clear about what qualities or traits are important to collect or build to look for comparisons or relationships. Adding a single variable can add significant cost, effort, and time spent on research. Some variables are qualitative and easy to collect. For example, gender, age are called nominal variables. Height and weight are quantitative and are called measurement variables because they can be measured and expressed in numerical order. Quantitative variables are also known as ratio or range variables as they can be placed or compared on a numerical scale. In some cases, there are attributes that can be compared but not easy to measure. An example is comparing rich and poor farms; this is difficult unless all assets are converted into monetary terms, which is not easy to do and in real cases people might not bother to do it. It may not be clear how rich the group is compared to the poorer group. Such variables are generally classified as ordinal variables. Since statistical science deals only with numerical facts, all qualitative information, even if not measurable, must be converted into numerical form before analysis using ranks or assigned numbers for the group differing in certain characteristics.

A variable after enumeration is also called data or numerical fact, which can be a continuous or discrete sequence. For example, the weight of fish can be between 1 kg and 2 kg, for example; Since the weights of the fish are given as 1.4 kg, 1.9 kg, even 1.45 kg or 1.92 kg, these data are continuous data. 1 and 2 are counted as the number of fish in a bucket, so there cannot be 1.4 or 1.9 fish in the bucket.

Quantitative information is quite difficult to enumerate in many cases, so it must be done carefully. These attributes, or nominal categorical variables, are arbitrarily assigned numbers or ranks to present the group and enable analysis using statistical tools. For example, if 1 represents white and 5 represents red,

2, 3 and 4 can be given according to the degree of color between these two. Similarly, groups could be given numbers such as 10 for best taste and 1 for worst by taste panelists when comparing flavors of fish from species, sources, or cooking methods.

5.2. Variables Used in Aquaculture

There are a number of variables used in aquaculture research. For an aquaculture experiment, the main measurable variables are the number and weight of fish, fry or eggs (individual or collective), against which other parameters are calculated and used for comparisons. Parameters that use two or more measurable variables are often called derived variables. An example of a derived variable in aquaculture is the survival rate, or the number of animals that survived relative to the number originally stocked. This is one of the most important derived variables in aquaculture and other animal production systems. Other important derived variables are growth and productivity. For example, daily weight gain, In younger stages, most organisms grow exponentially. Therefore, the appropriate variable or parameter for growth is to report the specific growth rate as a percentage of the specific growth rate, which can be calculated using the natural log (Ln). On the other hand, relative weight gain (or loss) is often used to compare the growth of broodstock fish; this means that the percentage of weight gain or loss is measured against the original weight. It is important to understand that this gain or loss may not just happen on a daily basis. For example, a female fish can lose a lot of weight right after spawning, which means it can happen within 1 minute. Most organisms have three distinct growth phases that are normally explained by asymptotic function. These; phase 1; Young phase (Exponential growth increase), Phase 2 grow -out satge (Increases at a constant rate), Phase 3 Old phase (Increases, but increases at low medium). Therefore, it is important to have as many intermediate data points as possible so that the actual growth curve can be plotted and comparisons can only be made at the endpoint. Similarly, the productivity of a system or yield is often reported in terms of net fish yield, which measures increased growth per unit area or daily volume. In general, ton·ha−1·year-1 is used for larger culture systems (especially soil ponds), while kg·m−2·day-1 or kg·m−3·day-1 is used for smaller culture systems, such as tanks and cages.

Reproductive performance is expressed as the total number of eggs or offspring produced by each female. It is expressed as fertility. Total fertility is determined by estimating the number of eggs per female per spawning. However, it can be misleading as the sizes of females and their ovaries differ significantly within the same group. Therefore, relative fertility, i.e. the number of eggs per kilogram of female per spawning, is used as a parameter for comparisons. However, reproductive performance of collectively managed frequent spawners (group as experimental unit) should be estimated per unit time period, ie day, week or month, rather than laying; for example, the daily or weekly number of eggs, fry or fingers (collectively referred to as seeds) per female or per kilogram of female in tilapia. Because tilapia rootstocks are managed in groups and require a significant amount of space, their reproductive performance is often measured per unit area of the breeding system, ie the number of seeds per square meter per day (Bhujel et al., 2007; Bhujel, 2011).

Feed conversion rate, net protein utilization and other feed efficiency parameters are used for nutritional research. It is quite common to see the effect of nutrients on the quantity or quality of germ cells, eg. eggs/rubs and sperm by counting or measuring their length and/or diameter. Also, the rate of feed converted to egg biomass can be an important parameter if the aim of the experiment is to analyze reproductive performance. If this data is in percentage format, the data needs to be converted. However, qualitative parameters, e.g. color and shape are quite commonly observed but not analysed. Similarly, the taste of wild and cultured fish is comparable. Because these data are of a qualitative nature, statistical analysis is possible only when they are assigned or coded with numbers.

Many parameters are interrelated. For example, fish survival has an impact on daily weight gain, and both have an impact on net fish yield. Therefore, multivariate analysis is needed in such a case. However, most researchers analyze these parameters separately. Additionally, most aquaculture researchers work hard to collect water quality parameters such as water temperature, pH, dissolved oxygen, nitrite and ammonia levels, but these are only analyzed. In many cases, they can mask the effects of the application. They can be used as covariates because they cannot be controlled, also called noise factors, but they have significant effects on survival and many production parameters. Their effects can be separated if they are used as covariates during

analysis. It can increase the chances of determining the real effects of the application.

Normally, economic parameters are not used to make statistical comparisons between applications. It is considered one of the most difficult parts of the analysis, as it is quite difficult to keep a separate record for each repetition. Economic parameters only matter when there is a significant difference between applications. Normally, the economic figures are determined for the combined application (collectively for all iterations of the application) and compared between treatments instead of calculating separately for each iteration, calculating the variance for comparison in a statistical manner.

Fish sampling: A researcher can weigh the entire group (group weight per repetition) and divide by this number to estimate the average weight, or sample a specific number of fish to weigh individually and calculate the sample mean as a representative weight of the entire replica group. The first method gives a more accurate average; however, in most cases it is not possible to weigh all fish. In such cases, it is necessary to sample a certain number of fish. It is very difficult to sample live fish and it is difficult to determine whether a suitable random sample can be taken as fish move rapidly from one corner to the other. Test units, e.g. soil ponds or tanks are quite large, although it is difficult, it should not be forgotten that samples should be taken from the middle parts as well as from every corner. Most researchers perform interim samplings, but do not use interim sampling data and only use final or endpoint data for statistical analysis. If interim samplings are available, the data collected can be valuable and informative to see the trend over the period rather than just the outcome. In such a case, time should be considered as a whole (block) while performing statistical analysis. Therefore, separating the block effect will increase the reliability of the test or reduce the possibility of error.

Sampling of feed and feed ingredients: Sampling of feeds and feed ingredients is very difficult, especially if they contain a large volume. It may be easier to sample commercial feeds as each pellet or mass must be uniform. However, if samples are to be taken from homemade feeds, extra care should be taken to ensure that the samples are representative. If large volumes are to

be sampled, multistage sampling should be applied. Since storage conditions have an impact on nutrient loss, sampling should be considered and representative sub-samples should be taken from each piece, e.g. from the bottom of the pile, the middle and top of the piles, and similarly from dark corners and well-lit areas. If in such a situation it is necessary to compare the storage losses of different types of feed or ingredients, the factors can be considered as factors (block = a whole) when the samples are kept separate and the data are analysed. If the feed samples are to be analyzed after a few days, they should be dried and stored in deep freezers. While preparing for analysis, the feed samples should be well ground and mixed homogeneously.

Water sampling and monitoring: Environmental factors to consider are water temperature, dissolved oxygen (DO), pH, turbidity, chlorophyll-a, ammonia, nitrate and nitrite concentrations. Within a pool, these parameters fluctuate with the daily cycle. Monitoring of water quality is particularly important for temperature and DO during the experiment, between 06:00 and 06:30 for the lowest in the early morning and between 14:00 and 16:00 in the afternoon to record the highest levels. Most water quality parameters often vary with depth; therefore, sampling or measurement should be made at 20 and 50 cm depths. But if the aim is to see the change in depth, measurements can be made at 10 cm intervals. For representative sampling, a simple water sampler can be made locally using PVC tubing that can be sealed when the sampler is slowly lowered into the water to collect water from the entire column. Usually, 100-200 mL of water, which can be stored in small plastic bottles, is sufficient for one analysis, but if there are other parameters to be analyzed then 1-2 liters of water should be sampled from various points. However, many researchers are confused about the volume of water to be sampled. They may think that only 1-2 liters of sample may not be representative, especially when trials are conducted in ponds containing large volumes of water. Fortunately, the water is more homogeneous compared to the soil at the bottom, which differs even within a centimeter of distance.

Sampling of eggs, muscle, blood and others: Depending on the purpose of the analysis, egg samples, muscle, blood and other parts of fish should be stored in a deep freezer (-20°C) immediately after collection. Care should be

taken to ensure that these samples are representative of the population. For example, for lipid analysis, the sample should be preserved using chloroform:ethanol (2:1) and butylated hydroxytoluene to protect it from oxidation of fatty acids. Similar standard protocols should be used for other purposes. If samples need to be transported, they should be stored in a sealed ice box. If specimens are transported across country borders, a researcher may be required to have certain documentation, as they are considered living animal cells.

Samples of eggs, muscle, blood, and other parts are used to compare changes in chemical or nutritional composition, especially for laboratory analysis. Their composition can be affected by the same processing factor, which means they can have correlation. At the same time, the correlation between the treatment factor and the regression analysis and dependent factors indicates the correct analysis method. In such a situation, multivariate analysis is the right tool, rather than analyzing one parameter one by one.

6. SAMPLE SIZE (VOLUME/NUMBER)

Deciding on the size of samples, both in volume and number, is the most important and at the same time somewhat difficult task. Sample size has a direct impact on the amount of effort and research costs. Many researchers decide on a tentative basis, which in some cases results in too much work and in other cases eventually a shortage of samples. It is therefore recommended to predetermine the actual volume required for analysis. In addition, an additional volume of the sample, e.g. at least twice or more of the amount is taken and stored in suitable places and conditions so that if any equipment, electrical or anything else fails while samples are being analyzed, stock can be used for repetition. This is particularly important where there is a limited time or window of opportunity for sampling.

When sampling water, soil, muscle, feed and feed components for analytical purposes, a small volume is usually sufficient. For example, 5-10 g of each bait or its ingredients is sufficient for close analysis. Similarly, 100 mL of water is more than sufficient for most laboratory analyses. However, a researcher must ensure that these samples are truly representative of where they were taken. In these cases, multistage sampling would be the best method. The basic principle is to collect the largest possible volume in the first step, to

represent the entire water volume of a pond, tank or other experimental unit. At the same time, the larger volume will be less affected in case of any contamination during processing of samples during analysis or sample preparation.

In most cases, sample size can mean the number of animals or objects representative of the entire population of any given experimental unit. Most researchers usually decide by percentage, e.g. 20%, 10%, 5% or 3%. However, when there are situations where researchers have to make decisions about sample size from large enough populations, e.g. tens of thousands, hundreds of thousands, even millions, then the percentage formula is useless because even 0.1% would result in an unmanageably large number. Two methods have been found to determine sample size.

Undoubtedly, the researcher wants to determine the most accurate with the least cost. In other words, the desire of the researcher to reveal the most secure data will be limited to his possibilities. Various formulas have been developed in various sources to determine the sample size.

Some of the events observed in nature or in social and economic life are defined by proportional discourses. For example; In hatching of fish, hatching rate or rate, mortality (death rate), etc., are the success levels of students in a class or university. In order to decide how many individuals will be included in the sample in research on such events, the rate or rate of occurrence of the event should be known, designed and determined in advance.

Some observed events find discourse with averages. For example; The weight or length of the fishing line juvenile fish taken to the fattening pad is indicated by the average. On the other hand, the average income of the fishing enterprises engaged in purse seine fishing can be mentioned. While determining the sample size in the investigation of such events, it is necessary to know, design and determine the standard deviation (N) of all the juvenile fish that make up the main population, or of all fishing establishments engaged in purse seine fishing.

It is impossible to determine the sample size (volume), in other words, the number of individuals to be sampled, without knowing the frequency or rate (p) or standard deviation (S) of the event to be examined.

The important question that comes to mind at this point is how can these values regarding the main population be known at the very beginning of

scientific research? Indeed, if these values are the main point of the subject; From previous research, The assistance of experts, managers and scientists on the subjects that make up the main audience, It is obtained by designing or determining a small (key) study (keystady) for the main audience.

Numerical elements that should be determined subjectively and before the research by the researcher are Confirmed deviation (d) according to the frequency (rate) of the observation of the event or the mean, Confirmed fallibility (or confidence) level (α) (Elbek, et al. 1998).

Determining the sample size is important in terms of decreasing or increasing the margin of error in research. Therefore, in the sampling method, the question of how many samples will be taken to represent the population constitutes the crucial stage of the subject.

Determination of sample size or sample size is possible at certain stages. In this context; In making estimates and interpretations to be obtained from the samples, it should be stated how much margin or margin of error can be tolerated. A mathematical equation should be found for the sample size (n) by making use of the fact that the limits of the estimates are equal to the tolerance amount. For this, the sampling method must be measurable, that is, the variance must be obtainable. For example, it may be necessary to have a sample size so that the variance remains less than 5%. In this case, the equality will be $V(\bar{y})$ α 0.05 and (n) will be solved from this inequality, The parameter values (S^2, P) for the population must be known, if not known, they are estimated. In some studies, data can be obtained for subdivisions of the population. Also, the amount of error to be tolerated may be different for each subsection. In such cases, separate (n) values are obtained for each subsection and their sum gives the total sample size to be applied. In a study, more than one subject is often examined. If a separate tolerance amount is determined for each subject, a different (n) set of values is obtained. From these values, the most appropriate sample size is selected according to the purpose and resources. If the resources are available, the largest sample size can be suggested, and if unfavorable, the sample size for the most important issue to be resolved can be suggested.

Three rules are important among some rules in determining the sample size.

1. In terms of statistical reliability, the number of individuals in the sample should be at least 30.

2. As the sample size increases, the amount of error ($S_{\bar{X}}$) in reflecting the population decreases. In other words, the sample size is inversely proportional to the amount of error.
3. Thirdly, as a result of the sampling process, it should be known that the data obtained from the sampling will not completely reflect the main population, and this can only be revealed by a complete census. Undoubtedly, the main purpose in statistics is to carry out this work with the least error and the least error in the way from the sample to the main population. However, as a rule, the assumption should be made that a healthy sample reflects the population well.

In terms of statistical reliability, it was stated that the number of individuals in the sample (sample size) should be at least 30. Smaller samples can also be taken if the main population is not very large (close to 30 or several times).

The general principle in determining the sample size is a good approximation to the standard error of the environment. As it is known, standard error is a concept that decreases as the number of units in the sample increases. Undoubtedly, for a population that has not yet been included in the study, whose parameters are unknown and statistical analysis has not been carried out, it cannot be directly possible to detect the error in a possible sampling. However, the problem can be approached by accepting a level of error that has benefited from similar research or that the researcher can confirm.

In determining the sample size, many calculation methods and formulas are suggested, which vary according to the sampling method and the general method adopted in the research.

Considering the fact that there is a certain correlation between the sample size and the standard error, it is necessary to determine the accuracy (confidence limit) of the sampling against this margin of error, which was previously approved by the researcher.

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Numerical elements that should be determined subjectively and before the research by the researcher are; Confirmed deviation (d) according to the frequency (rate) of the observation of the event or the mean, Confirmed fallibility (or confidence) level (α), Confirmed confidence (or error) level (α) shows how likely the value obtained as a result of the sampling will be within the determined ranges.

For example; If a 5% probability is predicted (α= 0.05), it is pre-approved that the value to be found will fall between these limits 95%, in other words, with a 5% probability, it will go outside these limits. Of course, if α=0.02, the proportional discourse would be similarly 98% and 2%.

6.1. Simple method for sample size estimation

In this method, as stated by Knud-Hansen (1997), the sample size depends not on the size of the population but on the potential variance among individuals in the population and on the sampling method, the sample mean can be expected to be the population mean; that is, an acceptable difference between the sample mean ($\overline{X}$) and the population mean (μ). Using the following t-test equation, the sample size (n) can be estimated as:

$$t_{\infty} = \frac{(\overline{X} - \mu)}{\frac{s}{\sqrt{n}}} \rightarrow \text{Calculated n} = \left[\frac{(t_{\infty}.s)}{(\overline{X} - \mu)}\right]^2$$

When calculated with this formulation, the sample size is calculated. However, if some die or become unusable for other reasons, it is recommended to take a larger sample than this to ensure that at least the individual here stays in the end. For example, if 10% mortality in transit is common, then:

Actual sample size (n) = Calculated n + (10% × Calculated n)

6.2. Comprehensive method for sample size estimation

A more comprehensive method of calculating sample size is to use the power of the test. As with the first method, the standard deviation is obtained by preliminary sampling or taken from related studies and the minimum detectable difference is assumed. In this method, it occurs in the case of asserting it as if in fact, even though the finding does not support the claim. If β is the probability of making a Type II error, statistical power is $1 - \beta$; this is the probability of detecting a significant difference or correctly rejecting a false null hypothesis. The following equations described by Zar (2010) can be used to estimate the sample size for a sample t-test assuming a minimum acceptable level of statistical power, i.e. 0.80, and also back-calculate the power of the statistical test if the sample size is known.

$$\text{Sample size (n)} = \frac{s^2}{d^2} x \left(t_{\alpha,df} + t_{\beta,df}\right)^2$$

Here, n is the number of samples or replicates, s is the standard deviation of the sample from preliminary sampling or similar past studies, d is the

minimum detectable or significant difference, df is the degrees of freedom, t_α is the df level of significance, and t_β is the df power of the statistical test.

Similarly, this equation can be used to determine the power of statistical testing.

$$t_{\beta,df} = d \div \sqrt{\left(\frac{s^2}{n}\right) - t_{\alpha,df}}$$

The power of the test is calculated by 1-β, where the value should be above 80%. If it is below this value, it means the sample size/repetition is less than necessary, which may result in the effects of an undetected/unreported application.

Similarly, for two-sample t-test and analysis of variance (ANOVA), sample sizes and statistical powers can be calculated using the following equations: For two-sample t-test:

$$n = \frac{2s_p^2}{d^2} x\left(t_{\alpha,df} + t_{\beta,df}\right)^2$$

Sample size and power of ANOVA:

$$\Phi = \sqrt{\frac{nd^2}{2ks^2}}$$

Here, Φ is the statistic based on seeing the probability from the F table, k is the number of operations/factor, d is the minimum detectable difference, and s2 is the variance.

6.3. Sample size estimation for survey research

The structure of the population included in the research is important in determining the sample size. The following formulas can be used to find the sample size, If the average of the event is to be examined; Formulas to determine the number of individuals to be sampled;

- If the number of individuals in the population is unknown,

$$n = \frac{t^2 S^2}{d^2}$$

- If the number of individuals in the population is known,

$$n = \frac{Nt^2S^2}{d^2(N-1) + t^2S^2}$$

n: sample volume, N: number of individuals in the population, t: Theoretical value found from the t table at a certain degree of freedom and detected error level, d: Deviation ± from the mean, S^2: Variance

If the incidence of the event is to be examined; Formulas to determine the number of individuals to be sampled;

- If the number of individuals in the population is unknown,

$$n = \frac{t^2PQ}{d^2}$$

- If the number of individuals in the population is known,

$$n = \frac{Nt^2PQ}{d^2(N-1) + t^2PQ}$$

n: sample size, N: number of individuals in the population, t: Theoretical value found from the table t at a given degree of freedom and a detected error level, d: Deviation to be made according to the incidence of the event, P: incidence (probability) of the event under investigation, Q: Frequency (probability) of the event under investigation.

In determining the sample size, the research method and design may require the use of different formulations according to different sampling methods.

For example; Calculations on how many animals should be included in the study and how many replications (repetitions) should be made between fish of a certain age and sex in which two processes known to be in close interaction with each other (for example, the drug) are applied, can be made with different methods (Baskan, 1998).

This method was proposed by Yamane (1967) for research based on social research. In this method, the sample size depends on the size of the population; however, the sample size does not increase in proportion to the increase in population size. Even if the population is very large, the sample size does not exceed 400; however, another factor affecting the sample size, II. The probability of making a type error (b) should be evaluated as 5% (90% confidence level) lower than the probability normally used, ie 10% in the equation.

Sample size equation (n) = N/ (1 + N × e^2)

Here, n: sample size, N: the total population and e is the probability of making a Type II error, or β (normally 10%).

For example, if a village has 400 fishing families, the required number of sample households is (n) = 400/ (1 + 400 × 0.102) = 80. In many cases, some families may be unresponsive or data may be missing, at least for some parameters. To compensate for this, 5-10% more should be considered for the survey. Therefore, the actual sample size is (n) = 80 + (80 × 10%) = 88.

Many researchers are confused about the sample size and the number of households to be surveyed or interviewed. Before making a decision, they should be clear about the research objectives that determine the number of samples and households needed. For example, if the aim of a survey is to compare the parameters of a particular village with national standard parameters established by the government or any organization (statistics bureau or similar) using standard methods, there is only one sample participating in the survey; therefore, the number of households to be surveyed is the sample size of that village as estimated above. However, if the researcher is to compare parameters between two villages, he must estimate the sample size for each village separately. This means that the total number of households to be interviewed is the sum of sample sizes that can double. Similarly, if the aim of a survey is to compare a parameter between two ethnic fishermen groups living in the same village, then a reliable source of information should be obtained or a preliminary survey should be done to know the approximate number of households in each. Thus, the actual sample size for each group can be calculated. In reality, it is unlikely that two ethnic groups will have the same population in one village or two separate villages. Therefore, the size of the samples will be different. However, most researchers try to equate the number either because they think it will be easier for data processing purposes or because they do not have statistical knowledge. If one chooses to use an equal sample size, then he or she should choose the highest number of villages surveyed.

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CHAPTER 10

THE IMPORTANCE OF ANESTHETIC USE IN AQUACULTURE AND ITS EFFECTS ON FISH WELFARE

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1. INTRODUCTION

Anesthesia is the applications performed in order to prevent the loss of consciousness and skeletal muscle reflexes and pain in the whole or a certain part of the body (Summerfelt ve Smith 1990; Küçük et al., 2016). Synthetic or natural substances used for anesthesia are called anesthetic agents.

Throughout history, people have sought various remedies to relieve pain and suffering. In this process, many different methods were used such as not only different kind of substances as opium and alcohol but also being knocked out by a blow to the head. The first application of modern anesthesia started in the 1800s when John Snow designed a mask and calculated the appropriate dosages of ether and chloroform (Bilgin, 2013). Also, the use of anesthesia in aquaculture started in the 1940s (Metin et al., 2018). Recent studies have shown that fish also experience pain, suffering and distress and respond to stressful and harmful stimuli (Sneddon, 2012). For this reason, appropriate use of anesthetic and analgesic agents is mandatory in order to alleviate or eliminate pain and suffering in processes that may cause pain or stress in animals and adversely affect animal welfare. This obligation, in addition to being an ethical and scientific obligation, also includes a legal obligation (Bronstad, 2022).

According to the procedure to be applied to the fish, anesthesia is performed as sedation, general anesthesia and intensive anesthesia (Table 1). While mild sedation is applied for calming purposes in short-term routine applications, deep anesthesia is used to minimize pain in invasive procedures such as surgical applications. Anesthesia levels were classified by different researchers based on balance and swimming activity, respiratory movements, heart rate in fish (Summerfelt and Smith, 1990; Coyle et al., 2004; Mercy et al., 2013; Küçük et al., 2016). Anesthesia response may vary by species, therefore species-specific behaviors should be considered when determining the level of anesthesia (Neiffer and Stamper, 2009; Güvenç, 2016).

Table 1. Anesthesia stages and monitoring findings

Stage	Level of anesthesia	Symptom
I	Light sedation	Reaction decreases to external stimuli, Equilibrium and respiratory normal
II	Excitation	Hyperactive behavior, Swimming activity and heart rate increases
III	Light anesthesia	Loss of muscle tone and equilibrium, slow and regular opercular movements
IV	Deep anesthesia	Loss of reflex against all stimulants, opercular movements and heart rate reduced.

1.1. Anesthesia Methods in Fish

1.1.1. Immersion anesthesia

Immersion is the most widely used anesthesia method in fish. The anesthetic agent is dissolved in water or a solvent and the fish are anesthetized in the bath method (Zahl et al., 2012; Güvenç, 2016). An artificial ventilation system is required for bigger fish or long procedures. The anesthetic agent is absorbed through the gills and skin of the fish and enters the bloodstream. Subsequently, anesthetic agents pass into the central nervous system (Neiffer and Stamper, 2009, Güvenç, 2016).

Water quality must be carefully controlled during immersion anesthesia. Some anesthetic agents can decrease the pH of the water, so the pH of the anesthetic should be checked and buffered if necessary. Dissolved oxygen and ammonia measurements should be monitored, especially in cases where a large number of fish are used or the anesthetic water is used repeatedly (Ross and Ross, 1999).

1.1.2. Parenteral anesthesia

Parenteral anesthesia can be administered orally, intracoelomic, intravenously or intramuscularly. The most commonly used forms of parenteral anesthesia are intramuscular and intravenous (Ross and Ross, 2008). In parenteral anesthesia, the dose is calculated according to the weight of the fish and the application is performed. Usually, parenteral anesthesia alone is not sufficient because anesthesia is also needed during weighing the fish or injecting the drug (Neiffer and Stamper, 2009). Therefore, parenteral anesthesia

can usually be applied after sedation with the immersion method. Compared to immersion, the effects of parenteral anesthesia are less predictable and may require aeration support due to long recovery time (Harms et al., 2005).

1.2. Ideal Anesthetic Selection and Preparation for Anesthesia

Before anesthesia, it is important to select the anesthetic agent and determine the concentration of anesthetic, to prepare the equipment and to ensure that the fish is suitable for anesthesia. The type and concentration of the anesthetic agent varies according to the physical and chemical quality of the water (temperature, pH, salinity, hardness, turbidity, etc.), type and size of the fish. Considering that the effect of the anesthetic agent is affected by a wide variety of factors, it may be advisable to test the concentration of the anesthetic in the pre-trial group before administration (Carter et al., 2011). The normal behavior patterns of the fish should also be known before anesthesia, such as caudal fin stroke rate, position in the water column, operculum movements. In addition, the personnel who will administer the anesthesia should constantly monitor the behavior of the fish under anesthesia and focus on the vital functions of the animal. Fish should be fasted for at least 12 hours before anesthesia and, if possible, monitored for 24-48 hours after anesthesia (Carter et al., 2011; Batt et al., 2005; Bronstad, 2022).

An ideal anesthetic substance should not have a toxic effect for fish, be environment friendly, be effective in less than 3 minutes, should not leave residue in the fish tissue, be easily available and economical (Ross and Ross, 2008; Marking and Mayer 1985).

2. ANESTHETICS APPLIED IN AQUACULTURE

In aquaculture, fish are subjected to routine aquaculture practices such as inspection, measurement, transport, vaccination, marking and surgery. While all these activities can increase the stress level in fish, they have important effects on fish physiology and survival (Harmon, 2009; Portz et al., 2006; Husen and Sharma, 2014). Stress affects the physiology of the fish, causing slow growth, suppressing immune function and inhibiting reproduction. As a result, all stress-induced physiological and biological changes negatively affect fish welfare (Barton 1997; Schreck & Tort 2016; Ciji & Akhtar, 2021). Deterioration of fish welfare directly affects fish health and growth, resulting

in economic losses (Ashley, 2007). Therefore, anesthetic agents are used extensively in aquaculture in order to minimize stress and eliminate sensitivity to pain. Appropriate sedation and anesthesia have the potential to limit adverse effects caused by the hypothalamic-pituitary-interrenal (HPI) axis, which plays a central role in the physiological stress response (Bronstad, 2022).

In addition to preserving fish welfare, using anesthesia to immobilize fish during difficult procedures such as blood sampling and spawning of large broodstock fish not only reduces the workload but also eliminates the potential risk of injury. Also, anesthetic agents are usually used in the transportation of fish both to reduce metabolic activity and to prevent mechanical injuries.

The most commonly used anesthetics in aquaculture are Tricaine methanesulfonate (MS-222), Benzocaine, Quinaldine, 2-Phenoxyethanol (2-PE), Metomidatei, Aqui-S and clove oil (Acherman et al., 2005; Aydın and Barbas, 2020; Ak et al., 2022). In recent years, researchers have been examining the anesthetic activities of herbal essential oils (Ak et al., 2022; Bodur et al., 2018; Can and Sümer 2019; Yiğit et al., 2022; Yiğit and Kocaayan, 2023). Since each species will have its own physiological and behavioral responses, the effective dose is as important as the anesthetic agent. In Table 2, the effective concentrations of anesthetic substances commonly used in aquaculture are presented depending on the species.

2.1. Tricaine methanesulfonate (MS-222)

MS 222, a benzocaine derivative, is powder and easily soluble in water (Maricchiolo and Genovese, 2011). It is commercially sold as Finquel and Tricain-S (Brown, 1993). Since MS 222 reduces the pH of the water, sodium bicarbonate or Tris-buffer are usually used for buffering. MS222 is excreted from fish in the urine within 24 hours and is almost zero at tissue levels (Priborsky and Velisek, 2018). The withdrawal period for fish to be consumed has been reported as 21 days by the U.S. Food and Drug Administration (FDA) (Ross and Ross, 1999). The effects of MS 222 on fish have been investigated in depth in the literature (Gomulka et al., 2008; Gholipour et al., 2011; Kristan et al., 2012; Fraser et al., 2014; Gressler et al., 2015, King et al., 2005). The most common side effects are changes in the biochemical content of the blood (such as hematocrit, hemoglobin, glucose, cortisol), osmoregulation and

disruption of ion balance, and cardiovascular effects (Fredricks et al., 1993; Di Marco et al., 2011; Gressler et al., 2015, Priborsky and Velisek, 2018).

2.2. Benzocaine

Benzocaine is a white, odorless, tasteless and water-insoluble anesthetic. Therefore, it should be dissolved in acetone or ethanol in anesthesia studies (Ross ve Ross, 1999). Benzocaine is used effectively at a concentration of 25-100 mg/L. Consumption of fish anesthetized using benzocaine has not been approved by the FDA (Coyle ve ark., 2004). Although benzocaine is cheaper than many anesthetics, long-term application causes side effects such as erratic swimming behavior on fish (Husen and Sharma, 2014). However, exposure to benzocaine does not adversely affect the growth or reproduction of fish (Ross and Ross, 1999). However, caution should be exercised in its use as the withdrawal period is not clearly established (Burka et al., 1997; Küçükosman, 2019).

2.3. Quinaldine

Quinaldine is a yellow-brown oily liquid substance. Like benzocaine, quinaldine must be dissolved in acetone or alcohol, because it is insoluble in water. Due to its cheapness, quinaldine is widely used in ornamental fish and sport fishing. Quinaldine, which is acidic, should be buffered with sodium bicarbonate (Coyle et al., 2004; Ross and Ross, 1999).

2.4. Clove oil

Clove oil is obtained by distilling the leaves, stems and flowers of *Eugenia aromatica* or *Eugenia caryophylata* trees. Clove oil is a brownish liquid substance with a pungent aroma and odor (Husen and Sharma, 2014). Although the active ingredient of clove oil is about 70-90% eugenol by volume, it also contains acetyl eugenol and turpenoid compounds (Küçük et al., 2016). Clove oil is less soluble in water, so it is dissolved in ethanol. Clove oil exerts its effect depending on Na, K, Ca and N-methyl D-aspartate (NMDA) receptor inhibition and GABA A receptor activation (Lee et al., 2005; Park et al., 2006; Güvenç, 2016). Clove oil has a short induction time and a long recovery time. The most important reason for this has been reported to be the long duration of

the effectiveness of clove oil due to its high lipid solubility and decrease in respiratory rate (Keene ve ark.,1998; Küçükosman, 2019).

Table 2. Usage doses of anesthetics commonly used in aquaculture according to species.

Anesthetic	Fish species	Size	Effective concentration	References
MS-222	*Oncorhynchus mykiss*	21 cm	60 mg/L	Sink et al., 2007
		169 g	100 mg/L	Yamamoto et al., 2008
	Acipenser persicus	30 g	150 mg L	Mohammadi and Khara 2015
		26 mg	75 mg/L	Falahatkar and Poursaeid (2017)
	A. ruthenus	811 g	5 mg/L	Bishkoul et al., 2015
	Sparus aurata	150 g	0,19 mM	Molinero and Gonzalez, 1995
Benzocaine	*Aulonocara nyassae*	1 g	75 mg/L	Ferreira et al., 2020
	Silurus glanis	45 g	100 mg/L	Gökçek et al., 2017
	Prochilodus lineatus	5 g	50 mg/L	de Medeiros et al., 2019.
2-PE	*Hypophthalmichtyhs molitrix*	72 g	500 mg/L	Hedayati, 2018
	Huso huso	190 g	700 mg/L	Shaluei et al., 2012.
	O. mykiss	13 g	0,30 ml/L	Velisek and Syobodova 2014
	A. gueldenstaedtii	5,8 kg	750 mg/L	Ak, 2022
Clove oil	*Psetta maxima*	100 g	180 mg/L	Aydın et al., 2015
	Garra rufa	1 g	50 mg/L	Aydın et al., 2019
	Dicentrarchus labrax	-	40 mg/L	Mylonas et al., 2005.
	Protopterus annectens	50 g	0,6 ml/L	Lederoun et al., 2019.
	Cyprinus carpio	-	50 mg/L	Husen and Sharma 2015.

3. EFFECT OF ANESTHETICS ON FISH WELFARE AND HEALTH

3.1. Hematological and Biochemical Effects

Anesthesia can activate the stress mechanism by inducing stress hormones due to hypoventilation (transient hypoxia) in fish (Sneddon, 2012). The occurrence of hypoxia increases cortisol and even lactate levels (Van Ginneken et al., 2004). Therefore, hematological and biochemical parameters are frequently used as stress indicators in fish (Fazio et al., 2017). Many studies have reported that anesthetic agents increase cortisol levels in several fish species (Boaventura et al., 2020; Davis & Griffin, 2004; Jia et al., 2022; Zahran et al., 2021). The use of clove oil as an anesthetic can often cause muscle numbness in fish, leading to hypoxia stress (Jia et al., 2022). Therefore, it has been proven by many studies that serum glucose increases with the application of clove oil (Josef Velíšek et al., 2011; Wagner et al., 2003). A dose-dependent increase in plasma cortisol and glucose levels was observed in *Sciaenops ocellatus* exposed to quinaldine for 15 minutes (Thomas & Robertson, 1991). Hematological parameters can be affected by processes such as transportation, exposure to pollutants, hypoxia, and anesthesia (Minaz et al., 2022). Routine hematological evaluation of fish includes definition of red blood cells (RBC), hematocrit (HCT), hemoglobin (HGB) and erythrocyte indices (mean corpuscular volume "MCV", mean corpuscular hemoglobin "MCH", and mean corpuscular hemoglobin concentration "MCHC"), and white blood cells (WBC) and thrombocyte counts (Campbell, 2004). Among them, WBC acts as a circulatory cell in the immune system (Magadan et al., 2015). Evaluation of eugenol and *Lippia alba* as anesthetics did not show an adverse hematocrit level for *Potamotrygon wallacei* (de Lima et al., 2021). In addition, no significant difference was observed for all other blood parameters (glucose, cholesterol, total protein, urea, lactate) both immediately and 48 hours after recovery, regardless of concentration. Exposure to anesthetic agents may also increase hematological variables (RBC, HCT, HGB) in different fish species (Gressler et al., 2014; Shaluei et al., 2012; Yousefi et al., 2022). In a study examining the first 16 hours after recovery, an increase in *O. mykiss* hematology parameters at the end of the first 8 hours, and a decrease after it was observed (Ak et al., 2022). It has been supported by several other studies that anesthetic agents increase blood parameters in the acute process after anesthesia (Gholipour

Kanani et al., 2011; Mohammadi & Khara, 2015; Uçar & Atamanalp, 2010; J. Velíšek et al., 2005).

3.2. Effects on Fish Histopathology

Histopathological examination is critical to confirm the response of organs, especially gills (main part for osmoregulation and gas exchange), to toxic or potentially toxic substances (Santos et al., 2020). The gills and skin are the first tissues to come into contact with the anesthetic in anesthesia studies applied through the bath (Gonçalves et al., 2022). Therefore, gill histology is an important indicator of the response to stressors (Fiedler et al., 2020). In a study examining the histopathological effect of essential oils, no histopathological damage was observed for the use of coriander as an anesthetic, while the use of thyme caused epithelial lifting in the gills (Yigit & Kocaayan, 2023). In another study, no histopathological findings of lavender and laurel essential oils were found in gill, kidney and liver (Yigit et al., 2022). The transportation study with clove oil as the most commonly used essential oil and alternatively tea tree for 15 and 30 hours did not cause significant changes in the gill epithelium (Santos et al., 2020). However, effects of clove oil on the gill epithelium such as hyperplasia and edema and fusion in the secondary lamellae have also been reported (Waristha et al., 2011). On the other hand, extract accumulation was observed in the gill tissues of clownfish treated with clove oil (Balamurugan et al., 2016). Higher gill tissue damage potential of clove oil for *Oplegnathus punctatus* highlighted compared to MS-222 (Jia et al., 2022). However, another study discussed the histological adverse effects of MS-222 on the gill tissues of Siberian sturgeon (Gomulka et al., 2008). For instance, it was determined that the use of clove oil in high doses caused vacualization, hyperplasia, and lamellar fusion in the gill tissues in Danube sturgeon (Ak et al., 2022). It has been observed that repeated applications cause mild gill necrosis in some species (Neiffer & Stamper, 2009). However, Velíšek et al. (2005), in an acute toxicity study with common carp (*C. carpio*), stated that clove oil did not cause permanent damage. In particular, anesthetic agent concentration, fish species and exposure time are stabilizing factors in the formation of histological damage. In a study designed with two different concentrations (10 and 15 mg/L) and two different essential oils (*Cymbopogon*

citratus and *Lippia sidoides*), the use of 10 mg/L *Lippia sidoides* did not have an irreversible effect on the gill tissue, while effects such as hyperplasia and aneurysm were observed in other options (de Oliveira et al., 2022). Similarly, *Lippia alba* and eugenol essential oil were compared and it was proved that especially the use of eugenol caused irreversible damage to the gill tissues of Potamotrygon wallacei (de Lima et al., 2021).

3.3. Anesthetics and Antioxidant Activity in Fish

Reactive oxygen species (ROS), also known as pro-oxidants, are compounds that damage proteins, DNA and fats (Evans & Halliwell, 1999). However, metabolism releases substances called antioxidants to reduce or prevent the oxidation effect of these oxidants (Halliwell and Gutteridge, 2007). As a result of this increase in the balance of pro-oxidant and antioxidant in favor of oxidant, oxidative stress occurs in organisms (Kisaoglu et al., 2013). Fish secrete enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) to maintain both this pro-oxidant/antioxidant balance and homotypemeostasis (Birnie-Gauvin et al., 2017). Oxidative stress promotes oxidative damage causing apoptosis and biochemical reactions that render cell function dysfunctional (D. F. C. Souza et al., 2019). The use of herbal anesthetics has been the focus of attention recently, especially due to its positive effect on antioxidant activity (Hussain et al., 2008). However, all anesthetics have the potential to induce oxidative stress, depending on the applied concentration, chemotype and fish species (D. F. C. Souza et al., 2019). For example, a low concentration of essential oil has the potential to suppress oxidative stress in silver catfish (Azambuja et al., 2011), while high concentrations caused oxidative stress (Salbego et al., 2014). Gill mRNA levels of gst3, the target gene for oxidative stress, increased after exposure to clove oil, but decreased to normal levels after recovery (D. F. C. Souza et al., 2019). Anesthetic effect of clove oil on *C. carpio* and reducing effect on SOD, GPx and glutathione reductase (GRx) in its brain and gill tissues have been reported (Josef Velíšek et al., 2011). In a study using *Melaleuca alternifolia* as an anesthetic, hepatic thiobarbituric acid reactive substances (TBARS) levels decreased, while an increase in GST enzyme activity was observed (C. F. Souza et al., 2018). The use of *Nepeta grandiflora* in the 10-hour tambaqui fish transfer process had a positive effect

on the antioxidant activity (Barbas et al., 2017). The use of 100 µl/L chamomile oil as a potential natural anesthetic on rainbow trout treated its antioxidant activity (Ak et al., 2022). On the other hand, antioxidant activity can be evaluated by feeding herbal anesthetics. In this context, SOD and CAT enzymes increased for Ictalurus punctatus fed 0.5 ml/kg Origanum vulgare (Zheng et al., 2009). Similarly, the use of *Cymbopogon citratus* (0.2 g/kg) and *Pelargonium graveolens* (0.4 g/kg) anesthetic agents as dietary additions increased CAT and GRx activities, but decreased malondialdehyde (MDA) levels (Al-Sagheer et al., 2018). In another study, feeding with diets treated with chamomile oil increased the antioxidant and immune response of shrimps (Abdel-Tawwab et al., 2022). In addition, antioxidant activity for synthetic anesthetic agents is worth examining. In rainbow trout muscle, intestine, brain and liver tissues, MS-222 has been reported to increase ROS generation and cause oxidative damage to lipids and proteins (Josef Velíšek et al., 2011). In another study, no negative antioxidant activity was observed on fish after anesthesia of benzocaine and MS-222 (Stringhetta et al., 2017). In a study investigating the use of 1,8-cineole at different concentrations (200, 400, 600, 800, 1000 and 1200µL/L),1000 µL/L anesthetic significantly increased SOD, GPx and CAT enzymes for *C. carpio* after anesthesia (24 h) (Hoseini et al., 2020).

3.4. DNA Damage and Genotoxicity of Anesthetics

DNA damage in aquatic organisms exposed to various materials (pollutants, anesthetics etc.) is determined by the comet test. The comet test is a simple, standard and rapid method used to quantitatively measure DNA damage in a contaminant-exposed organism (Bajpayee et al., 2005). Since genetic damage in erythrocytes of fish blood causes single and double DNA degradation, comet assay is used as a bioindicator against potentially toxic substances (Singh et al., 1988). Use of anesthetics for fish welfare may induce primary DNA damage and interfere with genotoxic effects (Gontijo et al., 2003). Therefore, genotoxicity tests on fish can provide a safety profile of the anesthetic agent for both the treated fish and the consumers (Kampke et al., 2018). Tricaine methanesulfonate (MS-222), which is approved by the FDA and is the widely used in fish, has been proven to have anesthetic potential in

Nile tilapia fish (Barreto et al., 2007). Also, MS-222 did not have a negative effect on DNA migration (comet tail moment) in this study. MS-222 has been accepted as a local anesthetic, although it is effective in fish (Braz & Karahalil, 2015). In another study examining different *Lippia alba* concentrations, no alkaline comet assay was observed for *Oreochromis niloticus* even at 300 mg/L concentration (Kampke et al., 2018). Similarly, the anesthetic effects and genotoxicity of eugenol, benzocaine and L. alba on O. niloticus and Astyanax lacustris were investigated (Nascimento et al., 2020). The mutagenic and genotoxic index of eugenol were higher for both fish species. The mutagenic index for A. lacustris did not significantly differ, while the genotoxicity index was adversely affected by eugenol. Although the mechanism is unknown, the genotoxic effect of eugenol in eukaryotic cells has been proven before (Cortés-Rojas et al., 2014). On the other hand, benzocaine did not show mutagenicity and genotoxicity index for *O. niloticus* and *A. lacustris*. However, significant differences were observed for commet tail length and tail DNA%. Benzocaine did not cause DNA migration on blood erythrocytes even over a 48-hour period. (Gontijo et al., 2003). *A. lacustris* essential oil as a natural anesthetic has been reported to be the most reliable anesthetic agent considering genotoxicity and mutagenicity indices and comet assay (Nascimento et al., 2020). In another study, it has been proven that the use of propofol as anesthetic does not have a genotoxic effect in case of low doses (Valença-Silva et al., 2014).

4. CONCLUSIONS

In this chapter, application methods and selection of anesthetic agents, determination of the appropriate concentration and its effects on fish are discussed for the use of anesthesia in fish. Anesthesia is a comprehensive issue that needs to be emphasized due to its effects on fish health, environment and human health, and ethical values. Determination of anesthetic is considerable due to fish species have different physiology and environmental requirements. In addition, it is necessary to examine the optimum concentration as the anesthetic efficacy may be affected by environmental conditions. It is important that the anesthetic agent is easily available and economical for the user, the application procedure is easy, and it does not cause any adverse effects for the consumer. Despite many scientific studies on anesthetics, the existence of suitable anesthetics in terms of human, environment and fish is still gap in the

literature. As a result, new studies are needed to determine the mechanism of anesthetic agents on fish, the appropriate anesthetic agent and concentration specific to each species and to reveal their possible side effects. In addition, it is necessary to develop the use of natural essential oils, which are also used for human consumption.

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CHAPTER 11

AN ALTERNATIVE FEED SOURCE FOR SUSTAINABLE AQUACULTURE: BLACK SOLDIER FLY (*Hermetia illucens*)

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1. INTRODUCTION

As the world population is predicted to reach 9 billion people by 2050, aquaculture development and growth are a global necessity to supply protein needs. Since 2015, aquaculture has supplied more than half of all edible aquaculture products (FAO,2020). In the previous 15 years, aquaculture has doubled. Since this situation, sustainable feeding of aquaculture fish become a necessity. Fish meal and fish oil production account for about 20% of total aquaculture production worldwide (Simke, 2019). In 2020, the global production was 5.4 million for fish meal and 1.29 million tons of fish oil (IFFO, 2021).

Aquaculture now plays a significant part in ensuring that people get enough protein while also consuming food that is safe to eat. Aquaculture's long-term viability is jeopardized by the high cost of fish meal, which is utilized as the primary protein source in feed.

If the fisheries and usage of species like anchovy, sardines, and mackerel in the production of fish meal and fish oil, which are used as basic raw materials in aquaculture feeds, do not decrease, other species that rely on them as food sources would become extinct. The path of aquaculture is wide open with decreasing of fish meal and fish oil in feed. The use of insect meal instead of fish meal, particularly black soldier fly (BSF) meal, has lately become one of the alternative possibilities (Simke, 2019).

BLACK SOLDIER FLY MEAL IN AQUACULTURE

In the aquaculture feed industry, it is very important to use protein sources as well as fish meal and soy meal. Scientists, fish feed manufacturers and politicians welcome the use of insect-based raw materials in aquaculture feeds to replace non-ruminant animal by-products (EU-regulation, 2013); Stamer et al. 2014). The black soldier fly, which can grow in a wide range of climate options, is seen as an important candidate in this field (Bondary and Sheppard, 1981). It has been observed that the protein content of the larvae, which can grow in many organic waste materials, can be obtained up to 42% and the oil content up to 35% (Sheppard et al. 1994; Stamer et al. 2014).

Black Soldier Fly (*Hermetia illucens*) attracts attention as a key feed raw material with the capacity to transform organic wastes in the environment

into a food source, can contain up to 64% protein in dry matter, and might be utilized in feeds instead of fish meal (Nauriti et al. 2021).

Insect meal as a protein source in feeds for the agricultural industry and husbandry has improved in recent years thanks to continued research and usage of insect sources (Sánchez-Muros et al., 2014).(Sánchez-Muros et al., 2014). Insects draw attention because of their short production periods and great nutritional content. (Khan, 2018). In terms of amino acid profile, it is regarded as a good raw material, comparable to fish meal and soy meal (Tran et al., 2015; Doan and Turan, 2021). The protein content of defatted BSF meal was determined to be 56.11 % dry matter, 4.86 % fat, 6.46 % moisture, and 11.39 % ash (Barragan et al., 2017).

Insect meal is regarded as a good alternative for fish meal in terms of protein and polyunsaturated fatty acid (PUFA) content, and it may be utilized in the feeds of a variety of fish species (Belghit el al. 2019). BSF meal has a similar amino acid profile to fish meal, has a high protein content (30-58%), and can be used as a healthy source of fat (St-Hilaire et al. 2007; Liland et al. 2017). They can also be used as a source of important n-3 fatty acids such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). In earlier studies, it was discovered that insect meal contains a protein level of up to 64% and a fat content of up to 30% (St-Hilaire et al., 2007). While BSF has significant levels of manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), phosphorus (P), and calcium (Ca), sodium and sulfur levels are reported to be lower than in other insect larvae (Wang and Shelomi, 2017). The alanine, methionine, histidine, and tryptophan content of BSF meal is higher than fish meal, but the methionine amount is lower. It has less arginine than soybean meal (Barragan et al., 2017). BSF larvae have 58-72% saturated fatty acids and 19-40% mono and polyunsaturated fatty acids, according to previous studies (Makkar et al., 2014; Surendra et al., 2016). Table 1 shows the fatty acid contents for BSF meal.

Table 1. Fatty acid profile of black soldier fry larvae

	BSF[1]	Fish Meal[2]
C12:0	53.81	0.09
C14:0	8.87	3.4
C:14:1n-5	0.15	-
C16:0	20.47	13.1
C16:1n-7	6.21	3.8
C18:0	4.65	18.9
C18:1n-9	23.37	6.0
C18:2n-6	0.13	0.6
C18:3n-3	5.17	0.5
C18:3n-6	0.15	0.1
C20:1n-9	0.27	0.6
C20:2	0.12	0.17
C20:3n-6	0.23	0.1
C20:4n-6	1.31	0.6
C20:3n-3	0.11	0.1
C20:5n-3 EPA	3.67	9.5
C22:2n-6	0.13	-
C22:6n-3 DHA	0.78	14.6
[1](Hender et al., 2021); [2](Glencross, 2020)		

Some processes such as hydrolysis, drying, and defatting appear to increase palatability, digestibility, and nutrient utilization (Newton et al. 2005). It is seen that the fat removal process is the most effective method, giving positive results in terms of essential fatty acid and amino acid balance, and the protein ratio can be up to 60%. (Henry et al 2015; Sprangers et al. 2017). In addition, it is thought that insect meal larvae meal can be an important source of fatty acids in aquaculture feeds, especially a supplement and alternative to algae-containing feeds that are rich in PUFA and EPA (Sealey et al. 2011; Liland et al. 2021).

Furthermore, substantial quantities of lauric, palmitic, and oleic acids have been found in BSF larvae (Surendra et al., 2016). BSF can be found in and around feces from animals, poultry, pigs, and humans (Dortmans et al., 2017).

They have also been found colonizing vegetable and fruit trash, municipal organic wastewater, and household and industrial garbage (Barragan et al., 2017). Larvae can be fed a wide variety of nitrogen and calcium-rich organic wastes in BSF culture units (Dortmans et al., 2017). Food with a moisture content of 52-70 % is preferred by BSF larvae (Shumo et al., 2019). Table 2 lists the nutritional contents for BSF meal.

Tablo 2. Biochemical content of BSF meal

Nutritional Contents	**BSF Meal**
Protein (% DM)	45.82±0.14
Lipid (% DM)	25.78±1.67
Moisture (% DM)	4.14±0.05
Ash (% DM)	6,85±0.34
NFE (% DM)	17.41±0.03
Alanine (% DM)	7.7
Arginine (% DM)	5.6
Histidine (% DM)	3.0
Isoleucine (% DM)	5.1
Leucine (% DM)	7.9
Lysine (% DM)	6.6
Methionine (% DM)	2.1
Phenlyalanine (% DM)	5.2
Serine (% DM)	3.1
Tryptophan (% DM)	0.5
Total MUFA	17.8
Total n-6 PUFA	10.6
Total n-3 PUFA	1.03
SFA-PUFA	5.88
Ca (mg/kg)	20.31±0.11
Fe (mg/kg)	0.249±0.10
Mg (mg/kg)	53.56±0.04
Mn (mg/kg)	20.03±0.01
K (mg/kg)	254.60±0.01
Na (mg/kg)	354.72±0.25
Zn (mg/kg)	0.24±0.01

(Barragan et al. 2017; Guerreiro et al. 2020; Zozo et al. 2022)

Furthermore, because BSF is neither a vector nor a pest, it is simple to grow in simple structures. It provides fish with prebiotics (Gariglio et al., 2019). When used in fish feeds, it lowers the cost of the feed while also promoting the growth of the fish, resulting in better resource management and profitability (Nauriti et al. 2021).

The protein content of BSF meal varies depending on how it is processed. Defatted BSF meal has a higher protein content than a non-defatted or partially defatted BSF meal (Cardinaletti et al., 2019). Degreasing is commonly done by freezing and cutting the larvae into little pieces (to allow the intracellular oil to drain out), then pressing under pressure for 60 minutes at 60°C, and then drying the material in an oven at 60°C for 20 hours (Kroeckel et al., 2012). It has been discovered that using defatted BSF meal in feeds makes grinding and pelleting procedures easier (Briggs et al., 1999).

Isolating chitin from protein is very important for improving the growth performance of animals given BSF larvae-containing diet. The separation of lipids from chitin is relatively simple since organic solvents easily recover the lipids. Alkaline extraction is used to separate the protein from the chitin, with 96 percent of the protein recovered (Caligiani et al., 2018). Table 3 illustrates the percentage of fish species that use BSF meal instead of fish meal or soybean meal.

Tablo 3. Summary of growth performance of different species of fish fed on BSF larvae meal diets.

Species	Substitute Level	FCR	Positive Effects
Rainbow Trout (Caimi et al. 2021)	Substitute 15% to fish meal	1.18	Positive effects on growth, feed utilization, whole-body composition, and FA profile.
Rainbow Trout (Dumas et al. 2018)	Substitute 13% to fish meal	0.91	The ADCs of protein and amino acid in BSF meal varied between 87 and 93%.
European Seabass (Abdel-Tawwab et al. 2020)	Substitute 50% to fish meal	1.42	The BSF meal inclusion in diets of European sea bass did not affect its body constituents. Additionally, values of red blood cells,

			hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were not significantly influenced by BSF meal inclusion in fish diets as compared to the FM-control fish.
Meagre (Guerreiro et al. 2020)	Substitute 30% to soybean meal	1.02	Overall, 10% of HM, corresponding to 17% of FM replacement, might be included in meagre diets without major adverse effects on growth, feed utilization, whole-body composition and FA profile
Nile Tilapia (Dietz and Liebert, 2018)	Substitute 100% to fish meal	1.80	Any negatitve effects on growth performance and improved the dietary protein quality of tilapia feeds.
Jian Carp (Li et al., 2017)	Substitute 100% to fish meal	1.52	Positive effects on antioxidant status of Jian carp.
African Cat Fish (Fawole et al., 2020)	Substitute 75% to fish meal	1.65	Impairing growth, nutrient utilization, antioxidant, and health status of the fish.
Barramundi (*Lates calcarifer)* (Hender et al., 2021)	Substitute 30% to fish meal	0.73	Improved bactericidal activity, immune-related cytokine expression, and mucin cells in both the gut and skin are an indication of improved immunity of barramundi.
Pacific White Shrimp (*Litopenaeus vannamei)* (Chen et al., 2021).	Substitute 20% to fish meal	1.55	Improve the intestinal microbiota without causing any negative effects

In recent years, improvements in BSF meal production procedures have proven more effective in particular fish species. The usage of 10-30 % in rainbow trout meals resulted in positive outcomes in terms of growth rate and fish health (English et al. 2021). The use of partially defatted BSF meal instead of fish meal in rainbow trout diets up to 15% without affecting growth performance, fillet physical characteristics, intestinal and liver health, or digestibility has been demonstrated (Caimi et al., 2021). According to Dumas et al. (2018), using up to 13% BSF meal in rainbow trout (*Oncorhynchus mykiss*) feeds improved growth performance. Abdel-Tawwab et al. (2020) found that using up to 50% BSF meal in European sea bass (*Dicentrarchus labrax*) diets had no harmful consequences. In meagre (*Argyrosomus regius*) diets, Guerreiro et al. (2020) found that using up to 50% BSF meal instead of soy meal had no negative influence on growth parameters. In Nile tilapia (*Oreochromis niloticus*) diets, Dietz and Liebert (2018) reported that using up to 50% BSF meal instead of soy meal had no negative consequences on growth.

Because of its high nutritional content in fish feeds and positive impact to growth rates, BSF meal has huge amount of potential. It is expected that using proper production and processing technologies (separation of oil and chitin removal), fish growth performance will be improved, expenses will be reduced, and pressure on aquaculture will be reduced, resulting in ecological and economic balance (Nauriti et al. 2021). It is predicted that the use of BSF meal in fish feeds will reduce and protect the species used to obtain fish meal. With the widespread use of insect meal in fish feeds, the protection of captured species will be ensured, especially to meet the need for fish meal.

It is known that chitin is a fiber that reduces the absorption of nutrients in the intestines and reduces the absorption and digestibility of lipids and proteins in fish. (Razdan and Pettersson, 1994; Han et al., 2009). Chitin has been shown to reduce the digestibility of lipids and inhibit nutrient absorption in the intestines. (Kroeckel et al., 2012). For example, Gopalakannan and Arul (2006) and Olsen et al. (2006), when included at 1% in common carp, tilapia and atlantic salmon feeds, were observed in feed intake and growth rates of these species (Nauriti et al. 2022).

2. CONCLUSION

It is expected that by using proper production and processing technologies (separation of oil and chitin removal), fish growth performance will be improved, expenses will be reduced, and pressure on aquaculture will be reduced, resulting in ecological and economic balance (Nauriti et al. 2021). Because of its high nutritional content in fish feeds and positive impact to growth rates, BSF meal has a huge amount of potential. With the widespread use of insect meal in fish feeds, the protection of captured species will be ensured, especially to meet the need for fish meal. It is predicted that the use of BSF meal in fish feeds will reduce and protect the species used to obtain a fish meal.

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CHAPTER 12

OXIDATION IN FISH FEED AND FEED INGREDIENTS

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1. INTRODUCTION

According to the United Nations population estimates, the world population is 7 billion 875 million people for 2021 (TUIK, 2021). Again, according to the estimates of the United Nations, the world population is expected to reach 8.6 billion in 2030, 9.8 billion in 2050 and 11.2 billion in 2100. On the other hand, it is stated that food sources will gradually decrease and especially protein sources will be insufficient. The main problem of our age is the protection and sustainability of food resources. Factors such as environmental impacts, climate change and global warming make access to agricultural food resources difficult. In addition, consumer awareness, healthy, adequate and balanced nutrition increase the need for animal proteins, and the perception that more animal protein consumption is the way to create societies with a high level of welfare is strengthened.

Fish meat, which is a valuable animal protein source, contains 17-21% protein. It is rich in vitamins and minerals. Fish oil consists of unsaturated fatty acids, especially ω-3 and ω-6 fatty acids. For these reasons, it is an accepted fact that fish and its products have a protective feature against cardiovascular diseases, high blood pressure and depression. In this respect, the importance of seafood, which is an important source of animal protein, is increasing, and it is considered as the sector of the future in world food platforms (FAO, 2018). However, aquaculture is encouraged in order to increase the production of aquaculture, as there is a decrease in the amount of production in natural fish stocks due to factors such as overfishing and pollution. The sustainability of aquaculture depends on the continuity of quality feed production. Researchers stated that feed input constitutes 30-70% of the unit cost in intensive breeding of carnivorous fish (Bostock, 2011; Özerdem et al., 2013). For this reason, it is necessary to monitor the quality of the feed. Feed selection and feeding management also have a significant impact on the economic performance of a production system (FAO, 2013). In this process, it is necessary to know the properties of the raw materials, which constitute the first stage of feed production, and to prepare a ration accordingly, taking into account the needs of the species (Bostock, 2011; Kop et al., 2019).

Many different raw materials are used in feed production. The nutritional content of these raw materials is checked at the purchase stage. These raw materials are then stored until feed production. Storage time and storage

temperatures directly affect the freshness criteria of feed raw materials. Especially when high-energy raw materials with high oil content are kept in bad storage conditions for a long time, the oils in the feed are oxidized, the number of peroxides increases and the feed becomes rancid. Peroxide is a term used for hydroperoxide compounds released by oxidation reactions. These compounds are powerful catalysts and accelerate oxidation reactions. As a result of oxidation, oils, vitamins and pigments are negatively affected and their bioavailability decreases. In addition, oxidized fatty acids react with lysine, negatively affecting the use of amino acids. Oxidized fatty acids also have a toxic effect. It has been reported that as a result of oxidation in fish feeds, slow growth, poor feed evaluation, discoloration, lethargy, fish mortality in long-term feeding are observed (Gao et al., 2012; Kubiriza et al. 2017; Liu et al. 2018; Yu et al., 2020).

2. FEEDS USED IN AQUACULTURE PRODUCTION AND NUTRIENT PROPERTIES

The aim of aquaculture farms is to obtain the highest quality product in the most economical way. For this, different types of nutrients (protein, fat, carbohydrate, vitamin-mineral) must be consumed by animals. Giving these basic nutrients to animals is through feed (Hoşsu et al., 2012). Feed; It contains organic and inorganic nutrients in its structure and when given in certain proportions, health, development, reproduction, yield, etc., they are foods that do not have a negative effect on their properties (Korkut et al., 2004).

It is not possible to feed by using a single raw material as feed in aquaculture. Because more than one feed raw material must be used in order to meet all the nutritional requirements of the living thing. For this reason, it is necessary to examine fish feeds in the group of compound feeds. Accordingly, Compound Feed; These are the feeds that are produced with a mixture of more than one feed raw material, consisting of organic and inorganic substances, whose structure is guaranteed, and which allows the grown creature to produce high quality and large quantities (Korkut et al., 2004; Kutlu and Çelik, 2010).

Vitamin-mineral mixtures and additives form the inorganic substances in the compound feeds, while the other nutrients (protein, fat, carbohydrate) contents constitute the organic part of the feed. In short, the feeds are in the structure of "organic matter + inorganic matter + water". The prepared feed

should contain at least 90% dry matter. Because protein, fat, etc., nutrients are contained in this dry portion. In general, it is not desired that the moisture (water) amount in compound feeds exceed 10% because it adversely affects the shelf life.

Feeding studies are not only aimed at gaining weight of the species. During the production period, all metabolic activities of the fish are regulated, brought to the marketing stage in the healthiest and fastest way, and even the treatment of diseases is provided by feed and feeding. Although there are not great differences between species in terms of nutrient requirements, there are significant differences between cold water and warm water fish, between freshwater and marine species. These differences are generally related to protein amount, essential fatty acids needs and carbohydrate use (Hoşsu et al., 2012; Tilami and Sampels, 2017).

Protein is an essential nutrient for growth and is often supplied from expensive raw materials. Insufficient or excessive use of protein in feed creates negativities in the growth and development of the fish. Fish meal is an indispensable protein source used in fish feeds because it contains a high level of protein, has a balanced amino acid composition and is found to be delicious by fish. The fact that the protein requirement in the feeds comes from fish meal at the maximum rate and the energy from fish oil at the maximum rate is very important in terms of digestibility, growth rate and FCR (Halver and Hardy, 2002; Korkut et al., 2004; Kop et al., 2019).

Carbohydrates are the first nutrients synthesized by photosynthesis in plants. The most common carbohydrates found in nature are polysaccharides. The most common types of these are starch and cellulose. The most difficult to digest carbohydrate type is cellulose. Carbohydrates are cheap and valuable energy sources for non-carnivorous fish and shrimp species. When used in feeds in appropriate proportions, they ensure that proteins are used for growth instead of providing energy. Carbohydrates act as a binder in the production of water-resistant baits. Some carbohydrates can enhance the flavor of feeds (molasses) due to their aromatic properties.

Lipids, namely fats, are an important source of metabolic energy (ATP) and have an energy of 9.5 KCal/gr. Like other living things, fish also need fats as a source of metabolic energy for growth, reproduction and movement (Hoşsu et al., 2012). In addition, fats form the structure of the cell membrane, and allow

vitamins A, D, E and K and pigments to dissolve in fat and pass through the intestine. Some enzymes are activated by lipids. In addition, fats are involved in electron transport in the mitochondria and in the transmission of information with neurons. Generally, most of the lipids in fish come directly from the feed. Lipids are the source of essential fatty acids that fish need for their development and health (Tocher, 2003). Fatty acids are divided into two classes, saturated and unsaturated. Saturated fatty acids are fatty acids with a single bond in their chemical structure, and unsaturated fatty acids are fatty acids with one or more double bonds. Unsaturated fatty acids are used by the organism for the production of some important biological substances. In case of deficiencies, significant problems are seen on growth (Bingöl, 1976; Hoşsu et al., 2008).

Fatty acids that cannot be synthesized by living things and must be taken with feed are called essential fatty acids. These are linoleic, linolenic and arachidonic fatty acids. According to their chemical structures, they are classified as n-3 fatty acids, n-6 fatty acids, and n-9 fatty acids. They are also called omega (ω) fatty acids. Unsaturated fatty acids get special names according to the number of carbon atoms and double bonds. PUFA to fatty acids with 18-20 carbon atoms and 2-4 double bonds; Fatty acids with more than 20 carbon atoms and more than 4 double bonds are called HUFA. Fish oil is the main source of oil used in aquaculture feeds. Polyunsaturated fatty acids (PUFA) are important in fish oil. Fish oil is also one of the most important dietary sources containing HUFA class omega 3 fatty acids such as EPA and DHA. While n-3 group fatty acids are essential for fish, n-6 group unsaturated fatty acids are also considered essential. It is very important to use essential fatty acids especially in female breeding fish and in the early stages of fish development. Nutrition with insufficient rations by essential fatty acids leads to significant developmental disorders in the embryo, decreased egg hatching rate and anomalies in the larvae (Hoşsu et al., 2012).

2.1 Ingredients Used in Fish Feeds

As a requirement of the digestive system and physiology of the fish, the raw materials preferred for fish feed should be of higher quality than the raw materials used in the feed of other living things. Especially their digestibility, amino acid and fatty acid contents, energy and starch values, mineral substance

groups stand out in this direction. In general, it is possible to group the feed raw materials used in fish nutrition as follows:

Commercial Feed Ingredients

a) Industry By-products

- Milling By-Products (Wheat, full fat soybean, rice, corn meal)
- Starch Industry By-Products (Wheat/corn gluten meal)

b) Fermentation By-Products

- Beer Industry By-Products (Brewer's yeast, malt)

c) Sugar Industry By-Products

- Molasses (as a binder and sweetener in the feed of herbivores)

d) By-products of the oil industry (Pulses)

- Soybean meal, Sunflower seed meal, Cottonseed meal, Canola meal etc.

e) Animal By-Products

- Milk and dairy products (Milk, Milk powder, other products of milk, Whey)

-Rendering (Meat meal, Bone meal, Meat-bone meal, Blood meal, Feather meal, Poultry by-product meal, Hydrolyzed Poultry Feathers, Chicken meal; ban has been imposed in EU countries and our country due to the risk of carrying various diseases.)

- Raw materials obtained from fishery products (Fish meal, Fish silage, Fish oil, Krill meal, Squid meal, Shrimp meal, Crab meal, Fish solubility)

f) Mineral Feed Ingredients

- Macro elements (Ca, P, Mg, Sodium salts)
- Micro (trace) elements (Fe, Mn, Cu, Ca, I, Mo, Zn, Se)

g) Additives

- Main foodstuffs (Vitamin/mineral premixes)

-Disease-protective or therapeutic substances (Antibiotics, Substances that prevent fat in the liver, Anti-toxins-Anti-mildew substances)

- Antioxidants (Etoxyquin, BHA, BHT, vitamins E and C)

-Substances that provide good evaluation of basic foodstuffs (Enzymes, Emulsifiers)

-Enhancing metabolic efficiency

- Substances that increase the quality of food

• Pigment substances (Astaxanthin/Cantaxathin Carotenoids)
• Binders
• Synthetic amino acids (Methionine, Lysine)

- Substances that increase feed consumption
• Taste and aroma substances

h) Single Cell Proteins (yeast, microalgae etc.)

In order to obtain a quality feed, it is absolutely necessary to choose good raw materials and to protect and control these materials very well, from the purchase of the fish to the feed (Alkan, 2012). High energy feeds are used in carnivorous fish production in temperate water conditions. The main energy source in fish feeds is oil. Fish oil is the main source of lipids used in feed. Soybean oil, hazelnut oil are alternative sources of oil that can be used in fish feeds. In addition, Meat-bone meal (10%), Fish meal (8.8%), Blood meal (6%), Chicken meal (14%), Spirulina meal (7%), Full fat soy (19%), Wheat red dog (5%), Wheat gluten (6%) are feed raw materials with high fat content (Hoşsu et al., 2012).

If high-energy feeds with high oil levels are kept in poor storage conditions for a long time, the oils in the feed are oxidized, accordingly the number of peroxides increases and the feed becomes rancid (Kop et al., 2019).

3. DETERIORATION OF LIPIDS (RANCIDITY)

Oils are easily oxidized and deteriorated due to the combination of unsaturated fatty acids they contain with atmospheric oxygen under the catalytic effect of heat, light and some heavy metals. As a result, there is a change in their smell and taste (Çakmak, 2003, Korkut et al., 2007; Hoşsu et al., 2008). Lipid oxidation is considered to be the main factor for deterioration in the quality of foodstuffs (Anwar et al., 2007).

Deteriorations in oils are examined in 4 main groups;

- Hydrolysis: The formation of a soapy structure, taste and odor in feedstuffs as a result of the formation of glycerol with free fatty acids.

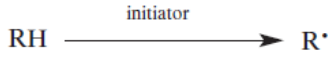
- Rancidity: bitter taste formation as a result of autoxidation of unsaturated fatty acids.

- Taste change: a change in taste caused by the oxidation of linoleic acids, especially in foods containing high levels of unsaturated fat, such as vegetable oils, fish oils.

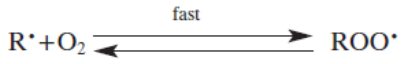
- Polymerization: It is the taste change that occurs as a result of the breaking of the chain between two carbon atoms (C-C), the formation of opposite bonds between two carbons or the formation of oxygen bonds in unsaturated oils (Çakmakçı and Gökalp, 1992).

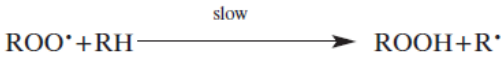
The oxidation mechanism of lipids includes three different stages. These are Initiation, Propagation, Termination stages. The steps generate different oxidation products (free radical mechanism, photo-oxidation and lipoxygenase activity) (Wasowicz et al., 2004). The first product formed by oil oxidation is hydroperoxide (Hamilton and Rossell, 1986).

1. Initiation Step: At this stage, one of the double bonds of the unsaturated fatty acid receives oxygen under the influence of heat, light and some metals, and free radicals begin to form. At this stage, there is not much change in the composition of the oils. The homolytic hydrogen atom leaves the methylene group and leads to the formation of the alkyl radical (R·).



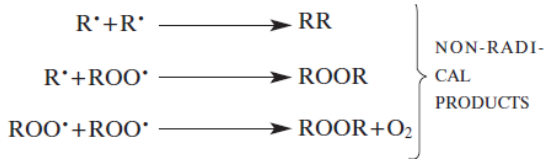
2. Propagation Step: At this stage, free radicals have started to form at critical levels and chain reactions accelerate. The rate of oxidation increases, oxygen begins to be absorbed rapidly and peroxidation is accelerated by the effect of free radicals. The formation of peroxide radicals (ROO·) can react with unsaturated fatty acids and form hydroperoxides.





3. Termination Step: In this step, the oxidation rate decreases. Free radicals combine with each other to form end products such as aldehydes, ketones, organic acids, alcohols, and hydrocarbons. It forms non-radical products with

the interaction of R· and ROO· (Wasowicz et al., 2004; Korkut et al., 2007; Saldaña and Martinez-Monteagudo, 2013; Tahir S., 2022).



3.1. Determination and Prevention of Oxidation in Oils

Various chemical and physical methods are used to evaluate the quality of oils and fat-containing foods (Wasowicz et al., 2004). In determining the quality of fish oil used as an oil source in fish feed production; Peroxide value, free fatty acid value, iodine value, moisture, total fatty acids, unsaponifiable matter, saponification value, titer value, residue can be interpreted by looking at parameters. The peroxide value in fish oil is very important and the acceptable value is 8-10 meq/kg (Boran et al., 2006; Kolanowski, 2010). Free fatty acids are fatty acids that are free in the oil and are not bound to glycerin, and a high concentration indicates that the oil becomes rancid. They reduce the utilization of the energy of the fat. The para-anisidine value (AV) refers to these high molecular weight carbonyl compounds (aldehydes, 2-alkenes, etc.). In an oxidized oil, peroxides are further converted to decomposed secondary species such as carbonyl. The maximum value of anisidine should be 20 meq/kg (NRC, 2002). Totox means total oxidation of oil. The sum of peroxides, aldehydes and ketones formed as a result of oxidation gives the totox (total oxidation) value (Korkut et al., 2007; Tahir S., 2022).

The oxidative state of oils is measured by determining the primary oxidation (peroxide value; PV), secondary oxidation (anisidine value, AV, thiobarbituric acid (TBA)) and total oxidation (TOTOX) values (Pignitter and Somoza, 2012; Semb, 2012). The primary and secondary oxidation values that it should have are accepted as given in Table 1.

Table 1. Primary and secondary oxidation values of oils

Parameter	Limit
Peroxide value (POV)	≤ 5 meq /kg
Para- Anisidine value (AV)	≤ 20
Total oxidation value (TOTOX)	≤ 26

(FAO, 2017).

4. EFFECT OF FEEDING WITH OXIDIZED FISH OIL ON GROWTH PERFORMANCE OF FISH

During the feeding of fish, it is inevitable that the oils undergo oxidation due to the heat, light and especially oxygen in the environment and accordingly the fish show nutritional problems. The reason why the growth of fish is reduced when they are fed with feeds containing oxidized oil is the deterioration of the synthesis and absorption metabolism of oxidized fats, as well as the negative effect of oxidized oil on the feed intake of the fish. In addition, the increase in fat concentrations in the liver is another factor that negatively affects growth (Luo et al., 2021; Yu et al., 2020; Dong et al., 2014; Chen et al., 2012).

The results of some feeding studies with oxidized oil on various fish species can be examined in Table 2.

Table 2. Effects of Oxidized Oils on Fish Species

Species	Oil Source and Oxidation Value	Duration (Day)	Body Weight Increase (BWG)	Feed Conversion Rate (FCR)	Survival Rate (SR)	Specific Growth Rate (SGR)	Hepatosomatic Index (HSI)	Condition Factor (K)	Reference
Atlantic salmon (*Salmo salar*)	-Herring oil/ POV: <1, 14 ve 40 meq/kg) - canola oil / (POV: <1, 5.5 ve 17 meq/ kg)	52	Negative		Neutral				Koshio et al. (1994)
Atlantic cod (*Gadus morhua*) juveniles	Fish oil / POV: 94 meq/kg + vitamin E	63	Neutral			Neutral	Neutral		Zhong et al., (2008)
Black sea bream (*Acanthopagrus schlegeli*)		63	Negative		Negative		Negative		Peng et al. (2009)
Japanese seabass (*Lateo labrax*) juveniles	Fish oil / soy oil 3:2, POV: 89.5 meq/ kg	56	Negative	Negative			Neutral		Zhang et al. (2016)
Yellow catfish (*Pelteobagras fulvidraco*) juveniles	Fish oil /1-2% oxidized oil	56	Negative			Negative			Zhuo et al. (2017)
Nile tilapia (*Oreochromis niloticus*) Arctic charr (*Salvelinus alpinus*)	Fish oil / 20.44, 182.97, 56.12, 33.27 ve 0.00 meq/kg^{-1} POV	90	Neutral Negative			Negative		Positive -	Kubiriza et al. (2017)
Channel catfish (*Ictalurus punctatus*)	Fish oil and soy bean oil / 0, 20, 40 ve 60 g/kg oxidized oil	56		Negative					Liang et al. (2018),
Largemouth bass (*Micropterus salmoides*)	Fish oil / POV: 7.2, 155, 275 ve 564 meq/kg	84		Negative		Negative			Yin et al. (2019),
Amur minnow (Rhynchocypris lagowski)	Fish oil / 0, 100, 400 meq/kg POV+ 500, 1000 mg/kg L-carnitine	56	Negative	Negative		Negative			Yu et al. (2020)

Nile tilapia *(Oreochromis niloticus)*	Fish oil / POV: 2.2 meq/ kg, POV: 120.6 meq/ kg + ferulic acid (0 - 400 mg / kg)	84	Negative	Negative	Neutral		Negative	Neutral	Yu et al. (2020)
Amur sturgeon (*Acipenser schrenckii*) juvenile	Fish oil / POV: 9.14 meq/kg POV:384.73 meq/kg	56			Neutral		Neutral	Neutral	Luo et al. (2021)
Hybrid grouper	Fish oil / 0, 3, 6, 9 % oxidized oil	65	Negative	Negative		Negative			Long et al. (2022)
Nile tilapia *(Oreochromis niloticus)*	Fish oil/ POV: 50.10 meq/kg and 100.80 meq/kg-1	84	Neutral	Neutral	Negative	Neutral	Negative	Neutral	Tahir, S. 2022

As seen in Table 2, the effect of lipid oxidation on the growth and physiology of fish varies according to different fish species. For example, Kubiriza et al., (2017) showed in their study that lipid oxidation decreased the growth of Arctic charr (*Salvelinus alpinus*) fish but increased the growth of Nile tilapia.

5. EFFECT OF STORAGE TIME AND TEMPERATURE ON VARIOUS OIL SOURCES

According to Boran et al., (2006) stored fish oils obtained from horse mackerel, shad, garfish and mullet at +4°C and -18°C. They evaluated the chemical quality of the oils with various parameters such as iodine, ester, acid, saponification, peroxide and thio-barbituric acid values and unsaponifiable matter at different time intervals for 150 days. According to the results, quality parameters of all oils except iodine and ester values increased at both temperatures during storage. It showed that oils stored at +4 °C retained their acceptable properties for 90 days, the acceptability tolerance was found to be 120 days for shad oil stored at -18 °C, and 150 days for mullet, garfish and horse mackerel oil. In general, it was determined that PV, TBA and AV increased gradually in all oil samples.

Anwar et al., (2007) investigated the extent of oxidative changes in soybean oil exposed to natural environment and sunlight for 180 days. Oxidative changes were monitored by periodic measurement of peroxide value (PV), color, free fatty acid (FFA) content, refractive index (RI), p-anisidine-, conjugated diene-, conjugated trienes- and iodine- values. At the end of the storage, the peroxide values of the oil samples stored in natural environment and sunlight reached 20.52 and 41.89 meq/kg, respectively. The results of the different parameters showed that the oxidative degradation of the oil samples stored in sunlight was significantly ($P<0.05$) meaningful compared to the stored oils in the medium.

Yıldırım (2009) observed the changes in chemical composition and oxidative quality of olive oils stored in the northern and southern Aegean regions for 14 months at room temperature and in the refrigerator. The results of the study showed that the oxidative stability of olive oil stored at room temperature is lower than that of oil stored in the refrigerator. Peroxides reached an acceptably high level (20 meq/kg) after 7 and 9 months of storage. It has

been shown that the increase in the anisidine levels of oils stored at room temperature is greater than those stored in the refrigerator. It was also observed that the oleic acid contents of both oil samples increased during the storage period, but the linoleic and linolenic contents decreased during the 12-month storage period.

Jaswir et al., 2009, evaluated the oxidative stability of fish oils obtained from 6 different marine fish species during 3 weeks of storage at -27 and +4°C. The results showed that the stability of oils stored at -27°C is better than oils stored at 4°C.

Flavia et al., 2014 investigated the quality of three types of animal fat (lard, beef tallow, and poultry fat) stored at +2°C and -18°C (refrigeration and freezing). Peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) in oils were determined to determine the primary and secondary oxidation state of the trial oils, and the profile of fatty acids and acidity value were determined to measure the degree of lipolysis in oils. According to the results, peroxide (PV) and (TBARS) values showed an increase at both temperatures during storage. It has been reported that fat samples stored at +2°C retain their acceptable properties up to 90 days in lard, 150 days in beef fat, and 60 days in poultry fat. In addition, the results of the study showed that the total content of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids decreased significantly in all oil samples after 210 days of storage at +2°C, and the acceptability tolerance of oils stored at -18°C, 210 days for beef fat, 150 days for lard and 120 days for poultry fat.

Almeida et al., (2019) samples of crude palm oil (CPO), refined palm oil (RPO), refined palm olein (RPOL) and refined palm stearin (RPS); They were stored under three different storage conditions in the dark (20-25 °C), refrigerator (4-8 °C) and room temperature (26-32 °C, exposed to natural light). In order to determine the stability of the oils, free fatty acids (FFA; %), peroxide value (meq O_2/kg), induction time (h), total carotenoids (ppm) and color (CIELab) analyzes were performed monthly for 12 months. The results determined that all crude/refined starting oils (CPO) were of good quality, while oil samples stored at 26-32 °C and exposed to natural light were subject to oxidative reactions. In addition, the estimated shelf life of CPO, RPO, RPOL and RPS samples stored at 20-25 °C and in the dark was approximately 6, 9, 9

and 12 months, respectively. Compared to oils stored under other storage conditions, they reported that the best quality oils were those stored at 4-8 °C.

Kop et al., (2019) investigated the effects of storage temperature and duration on the quality of oils obtained from various fish species and feed raw materials. Oil samples (Soybean oil, Anchovy oil, Salmon by-product oil, Norwegian origin fish oil, Salmon oil, Sprat oil, Black Sea Fish oil (anchovy + sprat oils) at room temperature (20°C) in laboratory environment, they were kept for 2 months under different storage conditions such as refrigerator (4°C), and 30°C ambient temperatures. The results showed that storage temperature has significant effects on the storage stability of fish oil, with fish oil samples stored at 4°C almost twice as long as those stored at 30°C. It has also been noted that oxidative and hydrolytic stability vary greatly depending on the fish species used to produce the oil.

Liu et al., (2019) investigated lipid oxidation and changes in nutrient content in peanuts due to storage. Peanuts were stored at different temperatures (15°C, 25°C and 35°C) for 320 days. In order to evaluate the lipid oxidation degree, the peroxide value (PV), carbonyl value (CV) and malondialdehyde (MDA) content of the oil obtained from peanuts were determined every 80 days. In addition, its chemical composition (fat, protein, total sugar, moisture and ash), fatty acid and amino acid compositions were also evaluated. The results of the study showed that the peroxide values of all oil samples stored at different temperatures increased gradually, increased faster at high temperatures (25°C and 35°C) than at low temperatures (15°C), and the fatty acid profile changed significantly during storage. In addition, it has been noted that long-term storage leads to changes in total unsaturated fatty acid content.

In their study, Yıldırım and Cantaş (2020) monitored the totox value in sea bass and sea bream feed for 45 days under two different storage conditions at +4 ºC and room temperature (+24 ºC) and aimed to determine whether it would be given to fish. As a result of the study, it was observed that there was no significant difference in terms of oxidation in fish feeds between storage at +24°C or +4 ºC during the first 30 days. However, totox values were found to be lower in feeds stored at +4°C in 45 days of storage ($p<0.05$).

6. CONCLUSION AND RESULTS

It is not easy to detect problems arising from feeding problems in fish production. In some cases, it may take months to see the negative effect. In the meantime, secondary results occur as a result of the decrease in the resistance of the fish due to the decrease in their feed utilization efficiency, and as a result, the pathogens in the environment make the fish sick. The manufacturer considers these pathogens as the source of the problem, but the real source of the problem cannot be considered to be from food. These are the situations that producers encounter very often or experience economic losses because of this (Tahir S., 2022).

Fish feeds are exposed to high temperatures (90- 150 ^{0}C) during their production. In this process, physical and chemical changes occur in the structure of raw materials. The feeds that become finished products are stored before going on sale. These feeds, which are sold to fish farms, are also stored here until they are used, and even under farm conditions, such as pools, cages, etc. They can stay under the sun and in wet environments in certain amounts before being given to the fish. In all these stages mentioned above, there is a risk of oxidation of the feed. Such losses that may occur in feeds in a long process and procedure during both feed production and fish production are generally not taken into account. However, the production and use of this type of feed is among the main causes of disease and death. In this respect, the oxidation mechanism and stages that may occur especially in high-energy fish feeds should be well known, the rotation of the feed should be followed, and attention should be paid to the storage and use stages of the feeds.

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CHAPTER 13

A REVIEW ON SOME TECHNOLOGICAL ADVANCES FOR INTELLIGENT AQUACULTURE

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1. INTRODUCTION

Aquaculture is a rapidly growing food sector, and its economic importance is constantly increasing (Yadav, 2022). Therefore, the contribution of aquaculture to world total fish production is indispensable (Adegboye et al., 2020). In the last 50 years, aquaculture production has significantly progressed, and the total fish production from aquaculture reached 87.5 million tons in 2020 (FAO, 2022). In this context, traditional aquaculture models have a crucial role in aquaculture developments and rapid production growth. However, as environmental awareness increased, various disadvantages of traditional aquaculture models gradually became apparent (Li and Li, 2020).

Further, a considerable increase in aquaculture, fish feeding, disease, and water pollution; moreover, causes many problems (Vidovic et al., 2014; Lafferty et al., 2015). Therefore, technological developments are needed to help overcome problems such as water pollution, disease outbreaks, fish-feeding, the quality of broodstock and fry, and poor management (Mustapha et al., 2021). Science applications and innovative technologies have recently supported aquaculture's rapid development and brought some solutions to these problems. Technological and scientific advances have benefited all aspects of aquaculture, and thanks to the benefits of technological development, the number of fish farmed has increased significantly.

Under controlled or semi-controlled systems, aquaculture production is conducted. Aquaculture has evolved from traditional farming to mechanized aquaculture and gradually to automated systems using innovative technologies (Li and Li, 2020). In addition, advances in automation and intelligent technologies have enabled aquaculture worldwide to gradually develop in an intensive and innovative direction, moving towards a sustainable aquaculture system and dramatically increasing aquaculture efficiency (FAO, 2018).

At the core of these technological developments, intelligent fish farming is a new field that aims to optimize the use of resources and promote sustainability in aquaculture (Ubina et al., 2021). Also known as the third green revolution, smart aquaculture aims to sustainably develop the aquaculture industry, increase production and be environmentally friendly (Vo et al., 2021). In other words, smart aquaculture can increase aquaculture efficiency, promote sustainable development, improve aquaculture quality, and minimize losses in the aquaculture process (Hu et al., 2020; Dikel and Öz, 2022). Intelligent

aquaculture focuses on decision-making quantitatively, data collection in real-time, AI control, precision investment, and personal service to create a new mode of fisheries production (Yang et al., 2020). The Internet of Things, Artificial Intelligence, Robotics, Drones, and Sensors improve the quality and efficiency of aquaculture. It is possible to make smart production and smart decisions using modern information technologies such as (Yang et al., 2021). For this reason, this brief review examines modern technologies related to smart aquaculture.

Internet of Things (IoT) and Aquaculture

The Internet of Things (IoT) refers to networks of physical objects that are constantly growing as IP addresses are connected and communications between objects and other devices and systems such as mobile phones/computers (Deekshath et al., 2018; Moparthi et al., 2018; Brous et al., 2019). In other words, the IoT is an internet-enabled network for smart devices that can collect and share information about usage and circumstances without interaction. The devices in such state use embedded systems via IoT like processors, hardware, and onboard sensors, and that means applying AI like machine learning for more straightforward data processing and better interpretation (Mattern and Floerkemeier, 2010; Dupont et al., 2018; Mustapha et al., 2021). IoT makes it possible for devices to communicate and communicate on a network and over specific infrastructures. It enables a new connection between computer-based systems and the physical world. This connection creates different possibilities. It enables advanced, efficient, accurate, and economic benefits in various fields (Yadav et al., 2022).

IoT has started to play an essential role in various industries (Gubbi et al., 2013). It is expected that with IoT-based technology, advanced services will be provided in land or water-based agricultural applications and all human activities in the coming years (Walter et al., 2017; Kamaruidzaman and Rahmat, 2020; Liu et al., 2020; Saiz-Rubio and Rovira- May 2020). As in other industries, information transmission technologies are much needed in the aquaculture industry to provide adequate connectivity with IoT devices, considering efficiency, reliability, accuracy, and secure network communication (Mustapha et al., 2021). Although IoT technology is new in aquaculture, it is developing rapidly, bringing new opportunities to the

aquaculture industry (Kamaruidzaman and Rahmat, 2020). Therefore, IoT-based aquaculture is a growing area of interest in the fishing industry (Jothiswaran et al., 2020). Real-time monitoring, forecasting, warning, and risk control are features of IoT applications in aquaculture. Wireless Sensor Networks (WSN) provides real-time monitoring capabilities and remotely manage systems as a critical component in IoT systems (Yadav et al., 2022). Together with WSN, IoT technologies enable the best use of resources to increase the efficiency of aquaculture farms (Motlagh et al., 2020; Gupta et al., 2022).

The use of IoT technology will bring several benefits to aquaculture farms. The installation of multiple underwater cameras and sensors in multiple cages for effective real-time surveillance and higher coverage is dictated by environmental conditions in aquaculture areas (Yue and Shen, 2021). It offers various solutions to monitor environmental parameters in aquaculture ponds and to determine and mediate appropriate solutions required by adverse environmental conditions (Geetha and Goutami, 2016; Saha et al., 2018; Tawfeeq et al., 2019). There are various components in IoT systems that enable these systems to perform many functions, such as identification, detection, application, operation, communication, and management (Ray, 2018; Yadav et al., 2022). Another thing is that with IoT, monitoring the environmental impact of fish farms in a sustainable and real-time way is also vital for better environmental management. IoT can also be applied to this end, which combines data over time with machine learning to create predictive models. There are elements in these predictive models that enable better and more accurate decisions thanks to the necessary warnings about potential risks. With big data solutions and IoT, many innovative steps can be taken that make the aquaculture industry more productive, sustainable, profitable, safer, and manage risk (Yue and Shen, 2021).

Aquaculture by Artificial Intelligence (AI)

Artificial Intelligence (AI)-based technologies are applied in many research areas related to the environment, sustainability, and climate sciences and attract increasing attention daily. The fundamental accepted paradigm of artificial intelligence is to solve problems by interpreting an automated intelligent task (Mustapha et al., 2021).

In recent years, in line with the increasing demands, the artificial intelligence industry has developed rapidly, and its use in aquaculture has increased. However, AI technology has not yet been widely adopted in aquaculture (Yang et al., 2020). Besides, for trouble-free and accurate results, equipment used in aquaculture systems needs an intelligent support system (Rembold, 2020; Wang et al., 2021). Moreover, although robots, drones, and sensors enable fast and real-time data collection, it is still exceedingly difficult to make accurate decisions due to the large amount of data collected (Evensen, 2020; Jothiswaran et al., 2020; Yue and Shen, 2021).

It is known that several research institutes and aquaculture technology entrepreneurs are working to make more effective and faster decisions and conduct artificial intelligence studies (Evensen, 2020; Razman et al., 2020). It provides optimal solutions to control AI, water production, and real-time factors in a highly dependable, accurate, and safe way for large farm applications. AI technology turns objects into devices that can learn and perceive. Therefore, collecting data and developing algorithms and networks becomes possible (Yadav et al., 2022). It is possible to use AI for aquaculture and computer technology to ensure operability in the aquaculture production process, monitor the development of underwater organisms, identify problems, and find solutions quickly, feed the organisms, cure diseases, and produce new descendants (Dikel and z, 2022). With artificial intelligence, aquaculture production can gain tremendous momentum, making aquaculture a less muscular field. Thus, AI helps to reduce maintenance and input costs and enable complete control over fish production systems. Input waste in aquaculture can be better managed with artificial intelligence, and costs can be reduced by up to 30% (Jothiswaran et al., 2020). In general, the five significant aspects of aquaculture, namely life information collection, aquaculture growth regulation and decision-making, fish disease prediction and diagnosis, and consideration of the aquaculture environment, are possible through artificial intelligence in aquaculture (Dikel and Oz, 2022). Many limitations still hamper AI due to the increasingly necessary limited amount of data. Because of this, it becomes essential for fish farms and large aquaculture companies to share their data in aquaculture production and marketing. Researchers and farmers will also benefit from a more extensive selection of sample data sets, sufficient data on the aquaculture production of each species under diverse cultural conditions,

and databases in public domains to develop advanced algorithms to make more accurate and better decisions (Yue and Shen, 2021).

Robotics in Aquaculture

In recent years, robotic technology has been widely used in aquaculture. For this purpose, many commercial companies and institutes have developed various aquaculture robots. Some strains produced have been evaluated and found to be effective (Yue and Shen, 2021).

Aquaculture is a complex process; many steps required for production are costly and labor-intensive (Lucas et al., 2019). It can be challenging to perform these steps without technological equipment. There are viable outcomes for these complex processes in aquaculture. Robotic systems, one of these solutions, can potentially do risky jobs in aquaculture. Robotic systems are an IT combination, machine, and automation. It may allow machines to participate in the entire fish production process just as humans have done for thousands of years (Rembold, 2020; Wang et al., 2021). It has been proven in many studies that robotic systems can be used in processes such as water quality monitoring and feeding automation, cleaning ponds and nets, injecting vaccines, and removing sick fish (Osaka et al., 2010; Lee et al., 2013; Luna et al., 2016; Antonucci and Costa, 2020; Sun et al., 2020). In addition, robotic systems can make aquaculture more profitable because robots can work continuously in bad environmental conditions without human assistance. Underwater robots, also known as uncrewed underwater vehicles, can perform some tasks underwater instead of humans.

An excellent example of this is netting in the salmon industry, where automated underwater robots monitor the current condition of the fish and clean their fish if necessary (Paspalakis et al., 2020). Another well-known fact is that robots are actively and effectively used to prevent and track fish health loss and farmed fish (Ohrem et al., 2020). Moreover, thanks to robotic systems, they have provided real-time monitoring of fish behavior (Kruusmaa et al., 2020).

Different companies have also developed commercial fish-feeding robots. Fish-feeding robots usually include a central computer and a robot for automatic feeding. The robot moves along its independent path and distributes pre-determined desired types of food to the tanks (Von Borstel et al., 2013).

It should be noted that despite the developments and diverse possibilities of fully automatic aquaculture robot systems, these will be exceedingly difficult to implement in practice and cannot be realized in the short term. Significant changes will be possible with the widespread use of robots and with the help of fish farming. On the other hand, any aquaculture automation that works in conjunction with robotic systems should be suitable for real-world culture systems because it is imperative to consider the specifics of culture systems, cultivated species, and different environments (Yue and Shen, 2021).

Drone in Aquaculture

Drones have enormous potential to monitor large areas quickly and efficiently. Like the robots mentioned above, drones can do much work above and below the water for the aquaculture industry. Drones collect aquaculture data more simply and quickly, which can help optimize farm production and management. For this reason, it has been frequently used in aquaculture. For this purpose, many commercial companies and institutes have developed and are developing distinct types of drones for aquaculture (Yue and Shen, 2021). Due to their information, studies on the use of drones in the aquaculture sector will increase daily.

Thanks to drones, aquaculture facilities and aquaculture fields on land and sea can be easily monitored. Many studies can be done with drones, including checking for holes and damage in cages (Sousa et al., 2019). For example, some drones can collect farm data, analyze fish stock, and track fish feeding data and changes in environmental conditions. These data obtained by drones can be easily applied in aquaculture studies. More importantly, drones can gather added information that is difficult for humans to obtain. In addition, drones can collect data that can help improve existing technologies and applications for aquaculture and offshore fish farming. This information and data can increase aquaculture production's efficiency and create algorithms for emerging technologies (Yoo et al., 2020; Setiyowati et al., 2022). In addition, drones provide a reliable surveillance system for aquaculture sites. Thanks to drones, suspicious objects that may cause potential security threats in aquaculture farms can be detected. It solves the complexity of fixed cameras installed to survey aquaculture fields and cages and gather the necessary information, and it can automatically perform the inspection task without the

complete supervision of the user (Ubina et al., 2021). Due to their information, studies on the use of drones in the aquaculture sector will increase daily.

Sensors in Aquaculture

Sensors are essential components that are small, easy to use, easily adaptable, have high network connectivity, and perform multiple functions (Basnet and Bang, 2018; Sharma and Dhenuvakonda, 2019; Li and Li, 2020; Yadav et al., 2022). Sensors play a significant role in the development of intelligent aquaculture. In recent years, the sensor industry has developed rapidly in line with the increasing demands, and its use in aquaculture has increased. Increasing demands for sensor technology have driven the development of new sensors. (Sharma et al., 2019). Most of the robots and drones used in the aquaculture industry use sensors to acquire real-time data in the water. With the development and application of new sensors in all aspects of smart aquaculture, activities such as field monitoring, remote debugging, remote diagnostics, and remote data collection will be possible.

Sensors can control dissolved oxygen (DO) levels, determine pH, and record water parameters such as salinity, turbidity, and pollutant concentrations (Su et al., 2020; Xing et al., 2019). Sensors must maintain water quality and develop early warnings of water pollution (Antonucci and Costa, 2020). For this purpose, biosensors are used in the aquaculture industry to detect dissolved oxygen levels and to analyze water salinity and temperature (Antonucci and Costa, 2020; Su et al., 2020).

In addition, using underwater sensors with an internet connection is an essential element to monitor the hunger and fullness status of fish growing in cages, ponds, and rivers, and fish can be fed according to the data from these sensors (Zhou et al., 2019). Appropriate feeding according to the organism's hunger-saturation state can significantly increase feed use, reducing feed waste. Thus, the overall production costs can also be reduced, and the cost reduction brings further positive results (Li et al., 2020; Su et al., 2020).

On the other hand, thanks to cloud computing and sensors connected to mobile devices, it can help maintain a suitable and ideal environment for fish. It can provide the aquaculture industry with optimal nutrition for growth and transformation (Yue and Shen, 2021). Some sensors access information via video surveillance and transmit it to systems using cloud computing methods

(Yadav et al., 2022). In the coming decades, developing a range of sensors to measure individual fish's stress level and detect pathogens in the water will be crucial and necessary. It will be important that the connected sensors can be easily placed in live fish or the water, and it will be inevitable that the devices can provide strong signals that they can detect on land, boats, or satellites (Yue and Shen, 2021). Therefore, controlled aquaculture production will contribute to the development of the aquaculture sector, and the production volume will increase even further.

2. CONCLUSION

This study aims to summarize some of the modern information technology and smart equipment used in various aspects of aquaculture. It becomes even more critical to monitor and control the production process to increase production in aquaculture facilities and improve fish product quality and animal welfare. The aquaculture growth rate will increase significantly with the continuous development of information technologies and the continuous improvement of intelligent and unmanned equipment. For this reason, technological developments such as AI, robotics, and IoT are increasingly applied in aquaculture production. On the other hand, intelligent aquaculture plans and forward-looking decisions are still more experienced-based studies than data. Therefore, intelligent technologies, equipment, sensors, and technologies to improve traditional aquaculture are required to realize intelligent aquaculture production (autonomous systems) and information management. It is also essential to sustainably develop an intelligent aquaculture model to integrate with information technology. To accommodate all these new developments in aquaculture, more data must be collected and used using advanced techniques to yield valuable results for future management and implementation decisions.

In this way, researchers may intervene in the aquaculture process in upcoming years and effectively prevent severe imbalances in water quality and aquaculture through wireless sensor networks, computer vision and image processing technology, IoT, AI, robotics, and many other technical procedures. It can improve production efficiency. It is already clear that interest in modern information technology and smart devices will soon increase.

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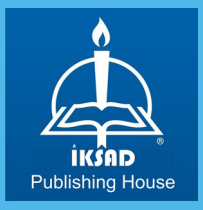

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