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RESEARCH ARTICLE

Proximate Composition, Trace and Macro Element, and Heavy Metal Content of Edible Seaweed *Solieria robusta* in Tawi-Tawi, Philippines

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ABSTRACT

Solieria robusta is a red seaweed consumed widely by the local population in the southern Philippines. However, current information regarding its proximate composition, trace and macro element, and heavy metal content is lacking. This study marks the first attempt in the Philippines to ascertain the proximate composition, trace and macro element, and heavy metal content of edible seaweed *S. robusta*. Our findings revealed that *S. robusta* primarily consists of ash content, measuring 44.30 ± 0.4 g/100 g, followed by carbohydrates at 32.96 ± 0.45 g/100 g. The moisture content of dried *S. robusta* was 16.91±0.04 g/100 g. The crude protein content of this edible seaweed was determined to be 5.61 ± 0.05 g/100 g, with total fat noted at 0.22 ± 0.01 g/100 g. The average concentration of trace elements (Zn, Fe, Mg, and Cu), macro element (K), and heavy metals (Pb and Cd) followed the order of Zn > Fe > K > Mg > Pb > Cu > Cd. Notably, three essential minerals -Zn, Fe, and K- were present in significant quantities in *S. robusta*, with concentrations of 219±8.70, 209±5.75, and 9.83±0.22 mg/kg, respectively. However, one trace element, Zn, and one heavy metal, Pb, exceeded the permissible values, and the rest were within the safe limits. This study constitutes a significant contribution to the comprehension of the nutritional profile and mineral composition of *S. robusta*, emphasizing its potential as a valuable dietary resource.

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1. Introduction

Seaweeds, commonly consumed in various cultures, offer a plethora of health benefits due to their rich nutritional profile. Packed with essential vitamins, minerals, and antioxidants, seaweeds contribute to overall well-being and can potentially support various bodily functions (Michalak & Chojnacka, 2018). They are particularly renowned for their high iodine content, which is crucial for thyroid function and the prevention of iodine deficiency disorders. Seaweeds also contain bioactive compounds such as fucoidans, which exhibit anti-inflammatory and anti-cancer properties (Sakthivel & Devi, 2019; Amlani & Yetgin, 2022). Additionally, the fiber content in seaweeds may aid digestion and promote a healthy gut microbiome (Praveen et al., 2019). Research suggests that the consumption of seaweeds may contribute to cardiovascular health by helping to regulate blood pressure and cholesterol levels (Cardoso et al., 2015). Furthermore, their potential anti-diabetic properties and role in weight management make them a valuable addition to a

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balanced diet (Chin et al., 2015). Incorporating seaweeds into one's regular nutritional intake could thus offer a range of health advantages, supporting both preventive and therapeutic aspects of overall wellness (Brown et al., 2014).

Solieria spp., a member of the Solieriaceae family within the Gigartinales order of Rhodophyta, exhibits a diverse habitat range spanning from marine environments to low-salinity estuarine settings. These red macroalgae, rich in carrageenans - linear sulfated galactans- hold considerable significance in various industries such as food, cosmetics, and pharmaceuticals, owing to their valuable properties as gelling, thickening, and stabilizing agents (Burlot et al., 2023). *Solieria robusta*, a seaweed that varies in color from yellowish brown to reddish, is typically found thriving in intertidal reef flats. It grows in rocky-sandy substrates and coral rubble and can even anchor itself to other marine animals, such as sponges (Ganzon-Fortes et al., 2006). As an edible seaweed popularly consumed by the Tausug and Sama/Badjao natives of Mindanao, Philippines, this seaweed is usually harvested from the wild and sold in local markets. It is commonly prepared and enjoyed as fresh salads, garnished with sliced onions, tomatoes, slivers of green mango, and a touch of vinegar (Tito & Liao, 2000). Furthermore, *S. robusta* possesses antifungal properties (Khanzada et al., 2007), which suggest its potential use as an organic fertilizer to address root diseases in crops (Sultana et al., 2011). In Tawi-Tawi, Philippines, the seaweed flora exhibits remarkable diversity, with 81 species cataloged to date (Puig-Shariff, 2015; Tahil & Liao, 2019; Dumilag et al., 2021). One of the edible seaweeds famous among locals is the *S. robusta*. Locally known as 'gulaman', this red seaweed is popularly sold in the local market and is known for its delicacy prepared as a salad (Dumilag, 2019).

Trace and macro elements play vital roles in sustaining health, participating in essential processes that keep the body functioning optimally. Macro elements like calcium, phosphorus, magnesium, sodium, and potassium are required in larger quantities and are pivotal for functions such as maintaining strong bones, supporting nerve function, and facilitating muscle contractions. These elements also contribute to energy metabolism and cellular communication. Conversely, trace elements like iron, zinc, copper, selenium, and iodine are necessary in smaller amounts but are equally indispensable. Iron aids in oxygen transport, zinc supports the immune system, and copper assists in wound healing. Acting as cofactors for enzymes, these elements ensure the smooth progression of critical biochemical reactions. Imbalances or deficiencies in trace and macro elements can lead to health complications, underscoring the importance of a balanced diet rich in these essential nutrients for overall health and disease prevention (Prashanth et al., 2015; Nieder et al., 2018; Skalnaya & Skalny, 2018). Heavy metals, crucial among inorganic pollution parameters, naturally occur in the Earth's crust and are non-degradable. While essential metals like copper and selenium are vital for human metabolism, they can become toxic when they accumulate in organisms beyond a certain concentration. Some heavy metals exhibit strong toxicity even in minute amounts and can be present in water as free ions, organic or inorganic compounds, or absorbed by particulate matter (Merian, 1991; Egemen, 1999).

Studies on the nutritional components, trace and macro element and heavy metal contents of edible seaweeds remain scarce. Therefore, this work determined the nutritional composition, trace and macro elements, and heavy metal content of edible seaweed *S. robusta* obtained from Batu-Batu Public Market in Panglima Sugala, Tawi-Tawi, Philippines. To the best of our knowledge, the present study is the first to report the proximate composition, trace and macro element, and heavy metal content of edible seaweed *S. robusta* in the Philippines.

2. Materials and Methods

2.1. Study Site and Collection

The sampling site is the Batu-Batu Public Market (5°04'12'' N, 119°53'01'' E), one of the major public markets in Tawi-Tawi, Philippines. During the market operation, edible seaweed, *S. robusta*, was purchased in fresh state form from the vendor on August 2023.

2.2. Sample Processing and Drying

An edible seaweed sample, *S. robusta*, was transported to the Fish Processing Laboratory, College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography. These were washed carefully and dried under the sun for 3-4 days and were sent to DOST-Region IX at Zamboanga City, Philippines, for analysis. The drying process was necessary since the analyses of samples were done in a dried form, and the results were expressed as mg per kg dried weight.

2.3. Trace and Macro Element and Heavy Metal Determination

Different trace elements (Fe, Mg, Zn, and Cu), macro element (K), and heavy metals (Cd and Pb) of edible seaweed *S. robusta* (dried form) were determined through the flame atomic absorption spectrophotometric method with dry ashing digestion technique (AOAC, 2016).

2.4. Nutritional Content Analysis

The proximate composition (moisture content, crude protein, total fat, ash, and carbohydrates) of the edible seaweed (*S. robusta*) samples, which were in a dried form, was determined using AOAC (2016). The moisture content of the edible seaweed was determined using the gravimetric method (air-oven drying at 65 °C). Crude protein was investigated using the Kjeldahl method (Block digestion and steam distillation). Total fat was analyzed using the

Randall/Soxtec/Ether extraction-submersion method with acid hydrolysis. Ash content was determined using the gravimetric method (Furnace at 600 °C). Lastly, the carbohydrate determination was done by obtaining the difference of the above compositions from 100.

Figure 1. Wet and dried edible seaweed S. *robusta*.

3. Results

The nutritional composition of the edible seaweed (*S. robusta*) in the present study is depicted in Figure 1. *S. robusta* is mainly composed of ash content of 44.30 ± 0.4 g/100 g, followed by carbohydrates with 32.96 ± 0.45 g/100 g. The moisture content of dried *S. robusta* was 16.91 ± 0.04 g/100 g. The crude protein of this edible seaweed was found to be 5.61 ± 0.05 g/100 g, and the total fat was noted at 0.22 ± 0.01 g/100 g.

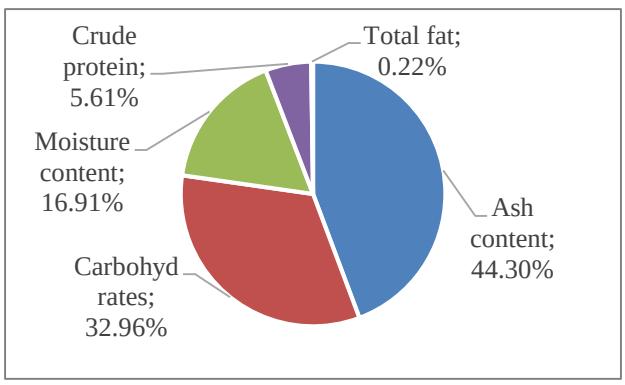

Figure 1. Proximate composition of edible seaweed *Solieria robusta* (dried sample).

The concentrations of determined trace and macro elements and heavy metals in the edible seaweed *S. robusta* are presented in Table 1. On average, the trace and macro elements and heavy metals are ranked in the following order: Zn > Fe > K > Mg > Pb > Cu > Cd. This implies that Zn and Fe are the most abundant trace elements in *S. robusta*. Specifically, the seaweed contains a significant amount of Zn (219±8.70 mg/kg) and Fe (209±5.75 mg/kg). Additionally, the K content was 9.83±0.22 mg/kg, while Mn was recorded at 5.95±0.36 mg/kg. Pb was present at 2.64±0.18 mg/kg, Cu at 1.55±0.02 mg/kg, and Cd was below the method detection limit. While the rest are within the safe limits set by FAO/WHO, one trace element, Zn, and one heavy metal, Pb, exceeded the permissible values.

Table 1. Trace element, macro element, and heavy metal content (mg/kg) of dried edible seaweed *Solieria robusta.*

Trace element				**Macro element**	**Heavy metal**	
Iron (Fe)	Manganese (Mn)	Zinc (Zn)	Copper (Cu)	Potassium (K)	Cadmium (Cd)	Lead (Pb)
209±5.75	5.95±0.36	219±8.70	1.55±0.02	9.83±0.22	<MDL	2.64±0.18

4. Discussion

Solieria spp., like other red seaweeds, are known for their high nutrient content. This makes them valuable in enhancing the nutrition of species like the olive flounder. Gunathilaka et al. (2021) showed positive results in their study on fish performance. Our research in the Philippines focuses on the proximate composition, trace element (Fe, Zn, K, and Cu), macro element (K), and heavy metal content (Cd and Pb) of the edible seaweed S. *robusta*. Since this is the first study on this topic, we could not make a direct comparison with existing research on the same species. Our findings broadly align with reported ranges for red seaweeds worldwide, often surpassing others, as detailed by Ullah et al. (2023). Various factors, including spatial and seasonal variations, geographical distribution, reproductive status, abiotic parameters, and species, influence seaweed's chemical composition, as discussed by Adharini et al. (2020). For instance, *S. filiformis*, a related species, cultivated in integrated multi-trophic aquaculture (IMTA), revealed water-soluble extracts with carbohydrate content ranging from 7.9% to 11.6%, protein levels between 12.3% and 12.8%, and lipids accounting for 0.67% to 0.72%, as documented by Peñuela et al. (2018). In comparison to other tropical edible seaweeds, our study found that *S. robusta* has lower protein content (5.61%), depending on geographical location. This falls within the range of 1.03% to 23.62% for *Kappaphycus alvarezii*. Additionally, it has less protein than edible *Caulerpa* species (which have between 10.41% and 21.52%), as reported by various researchers (Matanjun et al., 2009; Nagappan & Vairappan, 2014; Ahmad et al., 2016; Wahidatul et al., 2019; Zhang et al., 2020; Zuldin et al., 2021). Carbohydrates dominate as the primary constituent, making up nearly 33% of the composition in our study of edible seaweed *S. robusta*. Our results on carbohydrate content exceed those reported for the edible seaweed *K. alvarezii* (5-23%) by Suresh Kumar et al. (2015) and Adharini et al. (2020), yet fall short of the levels reported for edible *Caulerpa* species (37-50%) in prior studies (Matanjun et al., 2009; Nagappan & Vairappan, 2014; Ahmad et al., 2016; Zhang et al., 2020; Zuldin et al., 2021).

Trace elements (Fe, Mn, and Cu), macro element (K), and heavy metal (Cd) were within the safe limits of WHO, the US (EPA and FDA), and EMA. This indicates that the coastal waters of Tawi-Tawi are not heavily polluted by these elements and heavy metals, similar to findings reported by Imlani et al. (2022). However, one trace element, Zn, and one heavy metal, Pb, exceeded the permissible values set by FAO/WHO. As an edible seaweed, the high but safe content of Fe and K, with concentrations of 219±8.70 and 9.83±0.22 mg/kg, respectively, is beneficial for consumers, considering that locals have less access to these minerals from other sources rather than seafood. Regular consumption of seafood (e.g., edible seaweed *S. robusta)* that is rich in essential minerals like Zn, Fe, and K can have significant positive implications for human health. Zinc (Zn), a critical component for a robust immune system, aids in the activation and production of immune cells, bolstering the body's defense against infections. Additionally, it plays a pivotal role in wound healing and tissue repair, showcasing its importance in maintaining overall health (Prasad, 2008). However, precautions are necessary as the edible seaweed *S. robusta* contains an excessive amount of Zn that may be harmful with regular consumption. Elevated levels of zinc in the human body can lead to adverse health effects, including but not limited to gastrointestinal disturbances, such as nausea and vomiting, and interference with the absorption of other essential minerals like copper and iron. Prolonged exposure to high zinc intake may also compromise immune function and result in chronic conditions (Prasad, 2008; Plum et al., 2010; Wani et al., 2017). Therefore, it is imperative to monitor and regulate the intake of *S. robusta* to mitigate the potential negative impacts on human health. Iron (Fe), on the other hand, is indispensable for preventing anemia, as it is vital for the production of hemoglobin, the protein responsible for oxygen transport in red blood cells. Furthermore, iron is a key player in energy production, ensuring optimal metabolic function. For cognitive development, particularly in children, maintaining adequate iron levels is crucial (WHO, 2001). Potassium (K), another essential mineral abundant in seafood, plays a dual role in regulating blood pressure and supporting heart health. By counteracting the effects of sodium, potassium helps maintain healthy blood pressure levels, reducing the risk of hypertension-related complications. Moreover, it aids in normal muscle and nerve function, contributing to overall bodily well-being (Whelton et al., 1997). Embracing a diet that includes seafood rich in these minerals, such as edible seaweed *S. robusta*, can offer a multifaceted approach to achieving and sustaining optimal health. However, it is important to exercise moderation, as excessive intake of certain minerals can lead to adverse effects.

Furthermore, it is surprising that edible seaweed *S. robusta*, collected from the sampling site in August, contained a higher Pb content exceeding the permissible value. Pb is deemed the most significant toxic heavy element in the environment. When consumed above the limit, it has deleterious effects on humans,

such as respiratory issues, reproductive problems, neurological disorders, birth defects, cancer, and other adverse effects (Ara & Usmani, 2015). Generalizing the findings is still challenging because there are numerous sources of this edible seaweed supplying the market, and some consumers directly collecting from the wild may bring it immediately to their tables.

5. Conclusion

The consumption of *Solieria robusta* by local communities in the southern Philippines holds significant implications. As revealed by the present study, this edible seaweed offers a nutrient-rich addition to their diet, potentially addressing nutritional gaps. Essential minerals like Fe and K provide potential health benefits, supporting immune function and overall well-being. However, it contains higher values of Zn and Pb above the recommended limits. Additionally, recognizing the nutritional value of *S. robusta* may lead to economic opportunities through its cultivation. Sustainable harvesting practices can be implemented, ensuring long-term viability. Culturally, *S. robusta*, known as 'gulaman', carries important traditions and strengthens cultural ties within the community. In sum, this study's findings have broad-reaching impacts on the health, economy, and cultural heritage of the local population. Therefore, further studies need to be investigated as other consumers depend on different locations, and the month of the collection might also influence the overall nutritional and elemental contents of this edible seaweed.

Conflict of Interest

The authors declare no conflict of interest.

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RESEARCH ARTICLE

Women in the Gastronomy Arena: Gender-Based Experiences Encountered

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ABSTRACT

In this study, the difficulties experienced by female kitchen workers in their profession due to their gender were examined. In the research, which specifically questioned sexual harassment and glass ceiling syndrome in the workplace, a phenomenological approach was adopted and a qualitative research method was preferred. In this context, various questions examining the obstacles in question were asked to female employees employed in the kitchen departments of three different four- and five-star accommodation establishments operating in Kuşadası. The findings indicate that women are partially hindered due to their gender. In addition, three of the participants stated that they were harassed in various ways (verbal or sexual), and one of them stated that he witnessed it in some way, even though he was not harassed. In line with the results obtained from the interviews conducted with nine participants, various inferences were made and various suggestions were presented to sector representatives and senior managers.

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1. Introduction

While traditional gender roles have increasingly collapsed in recent years, significant changes are also taking place in the business world. As a reflection of this change, commercial kitchens are no longer places where only male chefs and cooks perform. Women leave their mark with their mastery and passion in these places where taste and creativity dominate. In a field where stereotyped norms and prejudices are challenged, the presence of women in kitchens not only affects food culture but also deeply affects social perceptions.

Kitchens, which were traditionally considered a male-dominated field, now attract attention with the mastery and creativity of women. Female chefs are responsible not only for their meals; they also leave their mark with their leadership. This situation deeply affects the understanding of taste and gender dynamics in kitchen environments. Understanding the factors behind the increasing participation of women in professional kitchens requires not only culinary culture; it also helps understand the evolution in the business world. The role of women in commercial kitchens is expanding the boundaries of the art of cooking while also challenging gender norms.

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Although sexual harassment at work is a common problem (Mohamad & Suhaimi, 2020), it can sometimes remain an unspoken problem for various reasons. Gender issues in commercial kitchens are compounded not only by food preparation skills and understanding of taste but also by gender identity. Sexual harassment against women in the business world affects not only individuals; it is also a problem that threatens the health of an entire organization. Harassment not only negatively impacts individuals' mental health but also has the power to shape the careers of female professionals and restrict their potential for advancement.

It's also possible that there is an actual glass ceiling syndrome in the kitchens of lodging establishments. This is because women are underrepresented in management, their administrative skills aren't properly evaluated, wage policies are based on gender, and job opportunities are shaped on a more fundamental level (Koç & Uşaklı, 2022). Cases of exclusion or harassment of female employees in the workplace due to gender frequently come to the fore. This situation creates concerns about women's entry or continuity into working life (Balkır, 2015; Öz et al., 2020).

In this study, the steps taken towards gender equality in the world of gastronomy and the challenges they face will be explored through the experiences of female employees in commercial kitchens.

1.1. Conceptual Framework

1.1.1. Gastronomy and commercial kitchens

Kitchens are designated spaces where the cooking process, often considered a form of artistic expression, takes place. These areas include the tools and equipment required for the preparation of food and beverages required for human nutrition. In addition, kitchens serve as areas where prepared foods are stored and, in some cases, facilitate the presentation and consumption of these foods (Aktaş & Özdemir, 2007).

Throughout human history, societies have engaged in eating and drinking activities. This situation has significantly affected the formation, structure, and organization of civilizations (Standage, 2018). Gastronomy is a combination of the Greek words gastro (stomach) and nomos (rules). Gastronomy is a branch of science that guides where, how, and when to consume food and beverages (Santich, 2004). In addition to meeting people's physical needs, food also affects their socialization, sensory and emotional states, and introduction to different cultures (Birdir & Akgöl, 2015). Gastronomy is not only the act of consuming food; it also includes preparation, cooking, presentation, and final consumption (Kivela & Crotts, 2006). Numerous definitions have been documented in the literature, from the beginning of the concept of gastronomy to the present day. Considering the similarities between these definitions, gastronomy can be described as a scientific and artistic discipline that deals with the preparation and presentation of food and is also a reflection of various cultures (Sarıışık & Özbay, 2015).

In the context of contemporary globalization trends, there is a remarkable shift in the approach of food and beverage businesses to provide services according to individuals' culinary preferences, social positions, and cultural backgrounds. These venues now prioritize the alignment of kitchen structures with specific thematic elements and invest significant financial resources to increase both the aesthetic appeal and functional efficiency of kitchen environments (Fine, 1996). With the industrial revolution and women's participation in business life, habits of eating outside the home or consuming ready-made food products have become common. This has enabled commercial kitchens to become widespread, thus creating a wide employment area.

Although the concept of a commercial kitchen often brings to mind kitchens in hotels, it is important to note that commercial kitchens cannot be limited to hotel businesses only. Sezgin and Ünlüönen (2011) classified cafes, cafeterias, restaurants, and similar institutions, especially restaurants, within the scope of commercial kitchen classification.

Individuals working in the field of cooking constitute an important component of the overall kitchen structure. The number, responsibilities, and qualifications of kitchen staff depend on several considerations, including the size, category, organizational framework, geographical location, physical configuration, and equipment of the establishment. Kitchen staff consists of many roles, such as head chef, section chefs, cooks, assistant cooks, dishwashers, and square attendants. The allocation of duties and tasks within the kitchen organizational structure depends on the unique roles individuals assume. Effective coordination and collaboration between kitchen team members is essential to optimize efficiency and ensure high-quality food production (Önçel, 2020).

1.1.2. Gender-based discrimination in business life

Gender-based discrimination has been a persistent problem that spans historical periods and is prevalent in contemporary society. Discrimination refers to any behavior that hinders individuals' entitlements, freedoms, and fair access to opportunities. Discrimination in organizational settings can occur as a result of demographic characteristics such as age, gender, and ethnicity, as well as other factors such as hierarchical placement and tenure. However, the type of prejudice that is most common and causes the most harmful consequences at the individual level is gender-based discrimination. Differential allocation of opportunities, resources, and incentives based on gender indicates the existence of gender-based discrimination (Yörük Karakılıç, 2019). Gender-based occupational discrimination refers to the perception that some occupations are inherently suitable for a particular gender. Therefore, it is seen that individuals' job

performances may differ according to gender. There is empirical data showing that throughout the job selection process, biases arising from social assumptions about gender perceptions of certain occupations play a significant role in perpetuating discriminatory practices. In addition, in the gender-based personnel selection process, not only the biological sex of the candidate but also their perceived masculinity or femininity levels are taken into account. Therefore, it can be argued that providing any data regarding a candidate's perceived gender has the potential to influence the assessment of the candidate's suitability for a particular employment position (Hareli et al., 2008).

Occupational discrimination is perpetuated through the use of sexist stereotypes, resulting in hiring choices based on the perceived suitability of a particular job for men or women. Therefore, gender-based discrimination can be among the determining factors in the recruitment decision process. The existence of gendered norms regarding paid work and domestic labor can lead to inequalities in the chances of men and women assuming senior management roles. Sometimes, women may be assigned lower salary levels than men. In this particular framework, the primary consequence of occupational segregation is the fragmentation of pay systems and the perpetuation of gender-based income inequalities (Kirshmeyer, 1998). The "alienation" of women may be one of the ways that gender discrimination is reflected in corporate culture. According to a study, in institutional environments where gender discrimination against women is common, women are treated as "foreigners" by men (Güler, 2005).

Gender discrimination is most commonly seen in employment, wage, and promotion situations. Some jobs are labeled as being for men or women by nature. This assumption is that one gender has only certain job-related characteristics, while the other gender lacks these characteristics entirely. In addition, prejudices against women in the workplace stem from the idea that women cannot prioritize their careers sufficiently due to family obligations or that they will work part-time jobs until they get married. In addition to discrimination in the workplace, prejudice can also arise when men and women are paid differently for doing the same job. Despite all their efforts, women may encounter a misleading glass ceiling that prevents them from advancing to senior management positions (Nasır, 1997). “Invisible woman syndrome” is a major issue affecting women in company culture. In male-dominated work environments, women are seen as domesticated, emotional, and irrational, while men are seen as possessive, controlling, rational, and logical thinkers.

As a result, women are perceived to be suitable for less valuable jobs than men in the organizational culture where non-organizational cultural values within the organization are maintained. Therefore, it can be said that due to this perception, female employees often find themselves working on low-level projects, and their opinions are not taken into account (Güler, 2005).

1.1.3. Workplace harassment

The United States is where the concept of “sexual harassment” first emerged. In 1975, American feminists were the first to use this expression. It quickly gained popularity, attracted media attention, and gained significant legal importance as a result of many court decisions. Later, it reached Europe and Japan, and over time, it became a current concept all over the world (Saguy, 2003). Sexual harassment is sexual pressure on a person to make sexual advances and requests against their will, and it occurs within an unequal power dynamic. According to Fitzgerald et al. (1995), sexual harassment is a behavior that involves three interrelated but conceptually distinct dimensions. These include gender harassment, unwanted sexual attention, and sexual pressure. Gender harassment includes a variety of verbal, physical, and symbolic behaviors that demonstrate a hostile and degrading attitude towards women. Sexual remarks or gestures, suggestive jokes, the display of sexual images, gender-based behavior, threats, or hostility are examples of such behavior that is intended to humiliate the woman rather than express sexual or romantic desires (Page et al., 2016). Unwanted sexual attention refers to unwanted, aggressive, and one-sided verbal and nonverbal behavior. Unwanted sexual attention consists of sexual expressions, intentional touching, aggression, and insistence on informal communication that the recipient perceives as unwanted, untrustworthy, and offensive. Sexual pressure is defined as the use of social power to achieve sexual intercourse (Herrera et al., 2018).

Physical, verbal, non-verbal, or visual behavior can all be considered sexual harassment. Looking intently or lustfully at someone's body is an example of nonverbal behavior. Rubbing or touching someone can be considered physical harassment behavior. Sexually explicit conversations are used to demonstrate verbal behavior, while using an offensive computer screen saver is an example of visual sexual harassment. In its most severe form, sexual harassment can take the form of rape or unwanted sexual jokes. The main feature of sexual harassment is that the person exposed does not want this action. Otherwise, laws against sexual harassment do not impede or restrict people's social interactions while they are living together (Yeşiltaş, 2005).

Sexual harassment is highly susceptible to individual perception, and many factors can influence how it is determined whether a behavior is abusive. Additionally, although men and women agree that behavior such as bribery or sexual assault is harassment, women are more likely to view these subtle behaviors as harassment (Golden et al., 2001). According to Eurofound's Fourth European Working Conditions Survey report, 10% of female employees in the Czech Republic, 7% in Norway, 6% in Turkey and Croatia, 5% in Denmark, Sweden,

Lithuania, and the United Kingdom, and less than 1% in Italy, Spain, Malta, and Cyprus reported having been sexually harassed (Unur & Şanlı, 2017).

Sexual harassment in the workplace creates an environment of fear and mistrust. Sexual harassment in the workplace must be addressed because it increases the victim's fear and causes serious physical, psychological, and emotional difficulties (Steiner & Wooldredge, 2015; Apell et al., 2019). Victims of sexual harassment often experience negative consequences such as resignation, absenteeism, interpersonal disagreements with co-workers, decreased work productivity, increased stress levels, and the intention to quit (Merkin & Shah, 2014).

1.1.4. Glass ceiling syndrome

Although there are many women employees at middle levels in organizations, the number of women in upper management is very low (Oakley, 2000). Looking at recent research on the lack of female employees in senior management, it is noteworthy that women are less represented in senior management, and even if they reach senior management, they are paid less than men (Pichler et al., 2008). Many studies have been conducted to determine the existence of the glass ceiling. Most research results have shown that female managers in the private sector only advance to the middle level and stay there. When looked at in terms of management levels, negative results are noteworthy in terms of the number of female managers. This supports the claim that there is a glass ceiling that prevents women from climbing the corporate ladder (Zel, 2002).

The concept of "ceiling" indicates that women face an upper limit on how high they can rise in the organizational ladder. The term "glass" describes the transparency and thinness of this barrier that the person cannot see (Barreto et al., 2009). The glass ceiling is defined as the obstacles that prevent women from reaching the most powerful, most prestigious, and high-paying jobs. The "glass" metaphor expresses an invisible barrier because there are no obvious obstacles to women reaching the top professionally (Longo & Straehley, 2008). The "glass ceiling" metaphor refers to the existence of an impermeable barrier that prevents women's vertical movements. Women can only be promoted below this barrier; they cannot go beyond it (Baxter & Wright, 2000). This situation creates organizational and perceptual barriers that prevent women from moving up the corporate ladder and reaching senior management (Weyer, 2006). Glass ceiling syndrome is a multidimensional phenomenon that includes organizational, gender, and social stratification factors (Kwaku Ohemeng & Adusah-Karikari, 2015).

The glass ceiling also draws attention to women's feminine qualities, such as being soft-spoken, loving, and compassionate. It can be stated that they cannot take a tough attitude like men in organizations (Adams & Funk, 2012). Fulfilling domestic and family obligations often creates obstacles for women. A large proportion of working women are single, divorced, or have fewer children as a result of their increased participation in employment (Guy & Schumacher, 2009). The social belief that men are smarter and more competent than women harms women's reputation in business life. In this context, the woman's colleagues or subordinates may also exhibit an attitude that questions this competence. In fact, in some societies, it may be considered humiliating or shameful for men to receive instructions from women (Connell, 2006). Women in senior management positions are also perceived as less proactive and less courageous. They are less likely to assume leadership positions within the organization and make ambiguous decisions (Bowles et al., 2007; Tan, 2008).

2. Methodology

In this study, phenomenology, one of the qualitative research methods, was preferred. This is due to awareness of the phenomenology involved. However, it focuses on facts about which there is no in-depth and comprehensive information. Phenomenology is a research methodology suitable for studies that aim to investigate situations that are neither completely unfamiliar nor predictable (Jasper, 1994). Rather than emphasizing the process of experience, phenomenology focuses on how individuals, societies, or communities convey the meanings they derive from their experiences and the meaning these experiences leave on people. It is the interpretation of the essence of events without limitation of space or time (Denzin & Lincoln, 1994). Qualitative research methodology was used to find in-depth and exploratory answers to the interview questions (Storey, 2007). The method used in the study was adapted from Arslan and Kendir (2020), Arslan et al. (2023), and Özçelik Bozkurt (2023). In this study, the potential difficulties that female employees of Kuşadası accommodation establishments may encounter in the work environment are examined, and how they make sense of these experiences is revealed. Each interview took place over the phone and lasted for about 10 minutes.

The questions asked of the participants within the scope of the research are as follows:

Q1. Do you think working in the kitchen is a disadvantage for women?

Q2. Do you think that your physical characteristics are a disadvantage when working in the kitchen?

Q3. Have you been verbally or sexually harassed at work?

Q4. Do you plan to continue in your profession?

3. Findings

3.1. Demographic Characteristics of Participants

The demographic characteristics of the participants in the research are listed in Table 1.

According to the information in the table, the participants are employed in different age groups and in different sections of the kitchen department.

Table 1. Demographic information of participants.

Participant	Age	Department Division
P1	23	Breakfast
P2	34	Hot Kitchen
P3	29	Hot Kitchen
P4	42	Cold Kitchen
P5	22	Breakfast
P6	35	Patisserie
P7	24	Patisserie
P8	23	Cold Kitchen
P9	25	Cold Kitchen

Table 2. Responses of participants to the questions.

	P1	P2	P3	P4	P5	P6	P7	P8	P9
Q1	No, I don't think gender affects professions. Everyone can do any job.	I think women's culinary disadvantages vary depending on the department. In my opinion, women manage the patisserie and men manage the hot parts.	I don't think women have a disadvantage in the kitchen, but I work in Turkey, and I think women are psychologically and physically abused.	I don't think there is a disadvantage for women working in the kitchen. I think there should be no gender discrimination.	Working in the kitchen can be a disadvantage for women. The businesses I work in have a large male population, so men see themselves as stronger than women.	I don't think so because, although the technology currently used reduces manpower, it makes work easier, regardless of men and women, and eliminates any disadvantages that may arise.	I don't think working in the kitchen is a disadvantage for women. Because professions have no gender.	I don't think working in the kitchen is a disadvantage for women.	I think there is a disadvantage for women in this patriarchal society, but it shouldn't be.
Q2	No. Because I think there is an equal and logical distribution of work in the kitchen, therefore my physical characteristics are not at a disadvantage.	There was no disadvantage for me in terms of physical characteristics in the kitchen, but I witnessed that my friends had problems.	Yeah, I think. If you are overweight, lose weight; if you were skinny, it was said to gain weight.	Yes, some of my physical characteristics had disadvantages. Since I am not very tall, I had difficulty reaching the shelves above.	I think my physical characteristics are disadvantageous from time to time. For example, not being able to reach the shelves above or carrying heavy things	No I do not think.	I think my physical characteristics are sometimes a disadvantage when working in the kitchen.	There are times when my physical characteristics are a disadvantage when working in the kitchen.	No I do not think.
Q3	No, I was not verbally or sexually harassed.	No, I was not verbally or sexually harassed.	Yes, I was verbally abused.	No, I was not abused.	I have not been verbally or sexually harassed personally in the work environment, but I have witnessed people being verbally harassed around me.	No, I was not verbally or sexually harassed.	No, I was not verbally or sexually harassed.	Yes, I was verbally abused.	Yes, I was verbally and sexually abused.
Q4	Because I love doing it, I plan to continue in the future.	I do not plan to continue in the profession. I turned to the teaching profession.	I plan to continue my career as an academician.	Yes, I am thinking of continuing in the profession.	I plan to continue in my profession despite everything.	I love my job and I plan to continue.	I love my job and I plan to continue.	I am thinking of continuing in the profession.	I am thinking of continuing my career as a teacher.

According to the data in Table 2, the first question was, "Do you think working in the kitchen is a disadvantage for women?" Three out of nine participants answered "yes," citing various reasons. Therefore, six participants think that working in the kitchen is not a disadvantage for women. The second question was, "Do you think your physical characteristics are a disadvantage when working in the kitchen?" Six participants answered “yes” for various reasons. Three participants stated that physical characteristics do not pose any problems for women in the work environment. The third question was, “Have you been subjected to verbal or sexual harassment in the work environment? One of the participants answered, "Yes, I was verbally and sexually harassed." Another two participants answered, "Yes, I was verbally harassed." Another participant answered, "I was not, but I witnessed my colleagues being verbally harassed." The fourth question was, "Do you plan to continue in your profession?" Three of the participants answered “no.” One of the participants responded, "I think of continuing despite everything."

4. Conclusion and Recommendations

In this study, which examined the difficulties faced by female kitchen workers, some participants stated that being a woman was not a disadvantage, while others argued that their gender was a disadvantage. In addition to the participants who stated that their physical characteristics pose a problem, especially in reaching high shelves, one participant pointed out that there were criticisms regarding their appearance. One of the participants stated that he was personally subjected to verbal harassment, and another participant stated that he witnessed the verbal harassment of other friends. The majority of participants stated that they were considering continuing in the profession. The fact that employees tend to stay in the profession suggests that the kitchen departments of accommodation establishments provide a more sustainable employment area compared to other departments. Because the general structure of the tourism sector has a structure in which the tendency to stay in the profession decreases over the years (Özçelik Bozkurt & Çökelsen Alkış, 2020).

The difficulties experienced by women due to their physical characteristics (not being able to reach high shelves, etc.) are not made to be felt as a deficiency by other male employees or superiors, directly or indirectly, which will ensure that women do not perceive this situation as a negative feature. This emphasizes that the struggle for gender equality is not limited only to social and cultural norms, but also includes elements such as physical infrastructure and ergonomics. Understanding these challenges and creating solutions demonstrates the need for a more comprehensive approach to gender equality in kitchens.

Participant responses show that the problem of sexual harassment in kitchens covers a wide spectrum. While participants who talked about their experiences of being harassed emphasized the seriousness of the problem in this area, witnessing shows that the problem is not only an individual but also a social issue. This diversity shows that the struggle for gender equality requires not only focusing on the individual's experience but also looking at the interactions around him from a broad perspective.

The occurrence of sexual harassment in the workplace causes significant health and psychological difficulties for affected employees, thus creating a challenging scenario for both individuals and businesses involved. As individuals' commitment to their jobs decreases, the level of competition in the sectors in which companies operate also decreases. Harassment significantly reduces individuals' job happiness and strongly affects their tendency to leave work. Additionally, as a result of harassment, individuals may experience psychological effects, including stress-related reactions such as anxiety and depression, as well as physical health problems such as headaches, gastrointestinal disorders, and sleep disorders (Fitzgerald et al., 1997). Previous research has revealed that female employees working in different branches of the tourism industry and in different positions are often subjected to systematic harassment cases and are exploited through pressure or in exchange for pay (Cheung et al., 2018; Ram, 2018). In their study on intern students, Unur and Şanlı (2017) found that male students were subjected to more harassment than female students.

This research offers important opportunities for accommodation business managers and relevant ministries to take effective steps in combating gender-based challenges. Hospitality managers should establish transparent policies and regularly update these policies to combat sexual harassment and gender-based barriers among their employees. They can also raise staff awareness by implementing training programs that support gender equality. Relevant ministries should inspect businesses in the sector and strengthen legal regulations on gender equality. They should also encourage businesses to take greater responsibility for addressing gender-based challenges in the sector by creating a comprehensive strategy. These efforts can make a significant contribution to creating a fair, equality-based working environment in the hospitality industry.

Conflict of Interest

The authors have no conflict of interest to declare.

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RESEARCH ARTICLE

Sensory Properties and Proximate Composition of Fish Soup from European Anchovy (*Engraulis encrasicolus* Linnaeus, 1758)

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ABSTRACT

This study investigated the proximate composition and sensory properties of fish soup prepared using European anchovy (*Engraulis encrasicolus*). According to the proximate composition analysis of fish soup, the crude protein, crude fat, crude ash, moisture, and carbohydrate values were found to be 5.04%, 6.22%, 1.46%, 82.05%, and 5.23%, respectively. In the sensory evaluation of fish soup, odor, oiliness, saltiness, bitterness, hardness, juiciness, aroma, appearance, overall acceptance, and purchase intent criteria were used. According to the sensory analysis results, it was seen that the panelists appreciated the anchovy soup. In the evaluation of overall acceptance, 46.66% of the panelists expressed "I liked it", 46.66% expressed "I liked it very much", and only 6.68% expressed "I liked it a little". Based on the expressed purchase intentions, it was unanimously declared by all panelists that they were inclined to get the fish soup. Considering the sensory analysis results, it has been shown that the fish soup obtained using anchovy can also be used industrially. Thus, these products are thought to contribute to increasing fish consumption and healthier food consumption.

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1. Introduction

Eating habits vary depending on the cultures that form the identity of a society. Although people's eating habits after birth vary over the years, it is one of the most important processes. After the first few years of ready-to-eat nutrition, the phase of consuming foods prepared in kitchens begins, and this phase continues throughout life unless something goes wrong. Culinary culture provides important information about societies in terms of eating and drinking. It is known that soup, which has an important place in Turkish culinary culture, is prepared from various fish and consumed with pleasure in the coastal regions of Türkiye. Soup is a primarily liquid food, generally served warm or hot that is made by combining ingredients of meat or vegetables with stock, milk, or water. Soup is an important food for healthy nutrition due to its nutritional properties, variety, taste, and juicy structure. Unlike other types of food, soups are not subject to influences such as seasonal characteristics, consumer age, and cultural differences in the preparation and consumption of soups (Köşker & Özbey, 2021; Özdemir Yaman, 2022). Soup, which has an important place, especially in the food culture of Turkish cuisine, has continued to be diversified and served on the tables from past to present (Çıtak & Sandıkcı, 2020).

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The limited food resources and the change in ecological balance in the face of the increase in the world population raise the problem of nutrition, which is the most important element of human life, especially healthy nutrition. The terms nutrition and especially healthy nutrition reveal accessing sufficient protein sources and taking them in an amount appropriate for the body (Caglak & Karsli, 2023). In order of importance as a protein source, animal proteins come before plant proteins. Aquatic products contain significant amounts of both protein and fatty acids (Öğretmen, 2022). Fish among aquatic products and anchovy among fish have an important population and economic value worldwide, as well as in Türkiye (Karsli, 2021).

Consumption and eating habits of anchovy fish appear as an important food phenomenon with cultural, economic, and sociological effects throughout history. There are many different recipes for anchovy fish, which is consumed in different ways of presentation (Yerlikaya et al., 2005; Kilinc, 2010; Üstündağ, 2010; da Silva et al., 2013; Çağlak et al., 2022). However, it is important to determine the sensory properties and nutritional quality parameters of these culinary preparations. In this context, this study aimed to prepare a soup using anchovy, which has a rich nutritional content, and to determine the proximate composition and consumer appreciation of this soup.

2. Materials and Methods

2.1. Materials

The anchovy used in the present study was obtained from a local fisherman and brought to the laboratory on ice in a styrofoam box. The ingredients in the recipe were obtained fresh from the local market.

2.2. Preparation of Fish Soup

Anchovy soup was prepared according to the recipe in the book "Anchovy from Sea to Table" by Çağlak et al. (2022). The ingredients and their amounts used in making soup are presented in Table 1.

2.2.1. Method

(1) After melting 1 tablespoon of butter in a pot, finely chopped onion and garlic were added and lightly fried. (2) Finely chopped carrots were added to the pot and roasted for another 2-3 minutes. (3) Potatoes cut into cubes were added to this mixture and fried for another 2 minutes. (4) Then, 2 tablespoons of flour were added and roasting continued until the smell of the flour disappeared and 7 glasses of hot water were added. (5) Bay leaves, celery stalks, salt, and black pepper were added, and the vegetables were cooked until they softened. (6) After cleaning the internal organs and bones of the anchovies to be used in soup making, the anchovy fillets were cooked in a pan until the water was absorbed. (7) Following the cooling of the cooked anchovies, 1 tablespoon of butter was added and lightly fried. (8) Finally, the cooked anchovies were added to the soup and served hot with finely chopped parsley (Figure 1).

Table 1. Ingredients and quantities used in soup preparation.

For soup		For sauce	
Ingredients	**Quantity**	**Ingredients**	**Quantity**
Anchovy (filleted)	300 g	Milk	1 cup
Butter	2 tablespoons	Egg yolk	1 piece
Medium-sized onion	1 piece	Lemon juice	2 tablespoons
Medium-sized carrot	1 piece		
Medium-sized potato	2 pieces		
Parsley	half bunch		
Garlic	2 cloves		
Hot water	7 cups		
Flour	2 full tablespoons		
Bay leaf	1 piece		
Celery stalk	1 piece		
Salt	4 teaspoons		
Black pepper	1 teaspoon		

Figure 1. Anchovy soup (original).

2.3. Analyses

2.3.1. Crude protein

Protein analysis was performed according to AOAC (1980; Method 2.507). In the total crude protein analysis performed according to the Kjeldahl method, 0.5 g of homogenized sample was placed in Kjeldahl tubes. 1 tablet (K_2SO_4+Cu_2SO_4) and 25 ml concentrated H_2SO_4 were added into the tubes as a catalyst and then placed in the Kjeldahl combustion unit. The sample was subjected to burning at 420 ºC for 5-6 hours until it acquired a green-yellow transparent color. After the burning process, 50 ml of pure water was added to the tubes that were left to cool, and they were distilled with 10 N NaOH and pure water. This process continued until the total volume reached 150 ml in a graduated conical flask containing 50 ml of 4% boric acid in the resulting distillate. Then, 10 drops of the indicator solution containing methyl red and bromocresol green were added and the distillate was titrated with 0.1 N H_2SO_4. The % crude protein amount was calculated according to the formula (1) below.

$$\text{Crude protein (\%)} = \frac{V \text{ x } 0.14 \text{ x} 6.25}{W} \quad (1)$$

Where; V is the titration volume (mL) of 0.1 N H_2SO_4 used, and W is the weight of the sample (g).

2.3.2. Crude fat

For the fat analysis performed using the extraction method, 3 grams of dried sample were taken in extraction cartridges, and placed in the extraction device. Diethyl ether (70 ml) was added into the tared glass crucibles and the samples were subjected to oil extraction for 110 minutes (Automatic Soxhlet Analyzer, Velp SER 148/6). The fat obtained from the sample was collected in a glass crucible and kept in the oven for 30 minutes to evaporate the diethyl ether. Then, glass crucibles containing fat were weighed and the % crude fat content was calculated according to the following formula (2) (AOAC, 1980, Method 2.507).

$$\text{Crude fat (\%)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Sample weight (g)}} \text{x} 100 \quad (2)$$

2.3.3. Moisture

Porcelain crucibles to be used in moisture analysis were dried in an oven at 105 °C for 2 hours and cooled in a desiccator. The tares of the cooled crucibles were taken and approximately 5 grams of sample was placed in them. Then, the crucibles were kept in an oven at 105 °C for approximately 24 hours until they reached a constant weight and were cooled in a desiccator. The cooled crucibles were weighed, and the moisture content was calculated with the following formula (3) (AOAC, 1995, Method 985.14).

Moisture (%) = [(Final weight– Initial weight) / Sample weight] x 100 (3)

2.3.4. Crude ash

For raw ash analysis, porcelain crucibles were burned at 550 ºC for 1 hour and the crucibles were cooled in a desiccator and tared. Approximately 2 g of the homogenized samples were placed into the tared crucibles. The crucibles were burned in a muffle furnace at 550 ºC for 5-6 hours and then cooled in a desiccator. Then, the cooled crucibles were weighed, and the % crude ash content was calculated according to the formula (4) below (AOAC, 1980, Method 7.009).

Crude ash (%) = [(Final weight– Initial weight) / Sample weight] x 100 (4)

2.3.5. Carbohydrate

The carbohydrate content of anchovy soup was calculated according to the formula (5) reported by Keskin et al. (2018).

Carbohydrate (%) = 100 - (crude protein + crude fat + crude ash +moisture content) (5)

2.3.6. Sensory analyses

Sensory properties of the fish soup samples were evaluated by a group of 20 volunteer panelists. Seventy-five percent of the panelists were male, and twenty-five percent were female. The average age of the panelists was established to be between 39 and 67. The evaluation was based on criteria such as odor, oiliness, saltiness, bitterness, juiciness, aroma, appearance, overall acceptance, and purchase intention. The form used for the sensory analysis of anchovy soup was revised from the MEB (2010) and Ulusoy et al. (2017) methods (Figure 2). Additionally, the purchasing intentions of the panelists were determined during the sensory evaluation.

Sensory Evaluation Form

Name:

Date: / /

Gender and Age:

Odor	**Oiliness**	**Saltiness**
1. Smell too strong to consume	1. Excessively oily	1. Too salty to consume
2. The smell is heavy	2. Too oily	2. Too salty
3. The smell of raw fish	3. A little oily	3. Slightly salty
4. No noticeable odor	4. Medium oiliness	4. Normal
5. Very faint smell of fried fish	5. Neither oily nor greasy	5. Slightly unsalted
6. Fried/steamed fish smell	6. Not oily	6. Too unsalted
7. Very distinct fried/steamed fish odor	7. Not oily at all	7. No salt
Bitterness	**Hardness**	**Juiciness**
1. Too bitter to consume	1. Too hard to beat	1.Extremely dry
2. Very bitter	2. Very Hard	2.Too dry
3. A little bitter	3. A little harsh	3.A little dry
4. Medium bitterness	4. Medium hardness	4.Medium dryness/wetness
5. Neither bitter nor not bitter	5. A little crunchy	5.A little watery
6. It's not bitter	6. Very crunchy	6.Very watery
7. It's not bitter at all	7. Super crunchy	7.Excessively watery
Aroma	**Appearance**	**Overall acceptance**
1. Not aromatic at all	1. I did not like at all	1. I did not like at all
2. Neither aromatic nor non-aromatic	2. I do not like	2. I do not like
3. Not aromatic	3. I neither liked nor disliked	3. I neither liked nor disliked
4. Slightly aromatic	4. I liked it very little	4. I liked it very little
5. Medium aroma	5. I liked it a little	5. I liked it a little
6. Very aromatic	6. I like	6. I like
7. In perfect aroma	7. I like it a lot	7. I like it a lot
*** Would you like to purchase this product?? Yes ( ) No ( )**		

Comments and suggestions::

Figure 2. Sensory evaluation form.

3. Results and Discussion

3.1. Proximate Composition

The proximate composition values of fresh anchovy and fish soup are given in Table 2. Crude protein, crude fat, crude ash, moisture, and carbohydrate values of fresh anchovy were found to be 16.80%, 7.20%, 1.53%, 73.52%, and 0.95%, respectively. A study was conducted to analyze the nutritional composition of anchovies, specifically focusing on Black Sea anchovies (*Engraulis encrasicolus*, Linne 1758) collected from the coastal regions of Türkiye, Georgia, and Abkhazia in the Black Sea. The study revealed that these anchovies exhibited similar levels of crude protein (16.1-17.9%), moisture (67.9-71.0%), crude fat content (8.23-12.2%), and crude ash (1.40-1.65%) (Öğretmen, 2022).

According to the present results, the crude protein, crude fat, crude ash, moisture, and carbohydrate values of fish soup were determined as 5.04%, 6.22%, 1.46%, 82.05%, and 5.23%, respectively. According to Kose et al. (2021), the values for moisture, crude protein, crude fat, crude ash, and carbohydrate content in the fish soup made from brook trout (*Salvelinus fontinalis*) were reported to be 87.79%, 8.18%, 2.89%, 0.62%, and 0.03%, respectively. Tufan et al. (2022) detected 7.22% crude protein, 87.56% moisture, 1.59% crude fat, 2.44% crude ash, and 0.07% carbohydrates in rainbow trout soup. Zhang et al. (2018) found that the moisture, crude ash, crude protein, and crude fat values of soups made from crucian carp (*Carassius auratus*) and snakehead (*Channa argus)* were 98.2-98.64%, 0.09-0.52%, 0.91-0.7%, and 0.17-0.13%, respectively. Compared to previous studies, it was determined that the moisture content of the anchovy soup in the present study was lower, and the crude protein (with the exception of Kose et al., 2021), crude fat, and carbohydrate contents were higher. It is thought that these differences may be due to the type of ingredients added to soup and their quantities, fish species, and cooking methods.

Table 2. Proximate composition of raw anchovy and fish soup (% wet weight).

Analyzes (%)	Raw anchovy	Fish soup
Crude protein	16.80±0.24	5.04±0.04
Crude fat	7.20±0.17	6.22±0.08
Crude ash	1.53±0.03	1.46±0.04
Moisture	73.52±1.86	82.05±0.82
Carbohydrate	0.95±0.05	5.23±0.12

3.2. Sensory Evaluation

Sensory analysis is one of the important criteria in the quality evaluation of seafood products, and criteria such as odor, color, texture, and appearance are evaluated by the human senses. For this reason, sensory analysis in quality control of seafood and other foods is of great importance for the consumer (Çağlak et al., 2015; Karsli et al., 2021).

In this study, an anchovy soup recipe was created to have a healthier and richer nutritional content. The sensory evaluation results of anchovy soup are shown in Table 3. Upon evaluating the odor criteria, it was determined that anchovy soup exhibited no adverse odor characteristics with regard to its consumability. In terms of odor, most of the panelists reported that they smelled “a very faint odor of fried fish” (53.33%), followed by “no noticeable smell” (40.01%). With regard to the oiliness criteria, most of the panelists (40.02%) stated that the anchovy soup was not oily. 26.66% of the panelists evaluated that the fish soup was “slightly oily”. The amount of salt used in the soup formulation was evaluated as “normal” by most of the panelists (86.68%). As determined by the bitterness values, the analysis revealed the absence of a prevailing bitter sensation in the anchovy soup, with a majority of the panelists (53.33%) selecting the “not bitter” choice. The hardness values of the anchovy soup were expressed by the panelists as between “extremely crispy” and “medium hard” criteria. Panelists expressed the juiciness of fish soup as “medium dryness/wetness” at a rate of 46.66%, followed by “a little watery (26.66%)”, “very watery (13.34%)”, and “excessively watery (13.34%)” options, respectively. The aroma characteristic of anchovy soup was expressed as “medium aroma” with 53.33%, “very aromatic” with 13.34%, and “excellent aroma” with 33.33%. The results of the anchovy soup in terms of appearance and overall acceptance were found to be similar, and 46.66% of the panelists chose the “I liked it” or “I liked it very much” options. After analyzing the purchase intent, all panelists claimed that they would be interested in purchasing the anchovy soup once it was made available on the market.

Some studies have been conducted in the literature on fish soup prepared using different fish species (Mol, 2005; Tolasa et al., 2012; Ulusoy et al., 2017; Zhang et al., 2018; Yavuzer, 2020; Kose et al., 2021; Tufan et al., 2022). Yavuzer (2020) prepared soup from the cooking water of different fish species (mackerel, bonito, and sea bass) and reported that all three fish soup groups received high scores according to the sensory analysis results. In addition, 80% of the panelists stated that there was no fishy odor/taste in the soup and 100% of the panelists who normally do not like to eat fish stated that they liked fish soup and could consume it regularly. In another study, Ulusoy et al. (2017) reported that adding monosodium glutamate to the low-sodium fish soup prepared from cultured sea bass improved the taste and flavor and that different flavors and additives could be used in product development.

When evaluated in general, even though both anchovy and different fish species were used, fish soup received high sensory appreciation from the panelists, as in this study. Soups are an indispensable product of the table with their quick preparation, economical, and satisfying properties. In this context, it is thought that the consumption of fish soup, as an alternative to the soups widely consumed throughout the world, especially in Türkiye, will make significant contributions in terms of both nutritional value and health.

Table 3. Sensory assessment results (%) of anchovy soup.

Evaluation Criteria and Results (%)					
Odor	**Results**	**Oiliness**	**Results**	**Saltiness**	**Results**
Smell too strong to consume	0	Excessively oily	0	Too salty to consume	0
The smell is heavy	0	Too oily	0	Too salty	0
The smell of raw fish	0	A little oily	26.66	Slightly salty	6.66
No noticeable odor	40.01	Medium oiliness	6.66	Normal	86.68
Very faint smell of fried fish	53.33	Neither oily nor greasy	26.66	Slightly unsalted	0
Fried/steamed fish smell	6.66	Not oily	40.02	Too unsalted	6.66
Very distinct fried/steamed fish odor	0	Not oily at all	0	No salt	0
Bitterness	**Results**	**Hardness**	**Results**	**Juiciness**	**Results**
Too bitter to consume	0	Too hard to beat	0	Extremely dry	0
Very bitter	0	Very Hard	0	Too dry	0
A little bitter	0	A little harsh	0	A little dry	0
Medium bitterness	0	Medium hardness	26.66	Medium dryness/wetness	46.66
Neither bitter nor not bitter	20.02	A little crunchy	26.66	A little watery	26.66
It's not bitter	53.33	Very crunchy	33.34	Very watery	13.34
It's not bitter at all	26.66	Super crunchy	13.34	Excessively watery	13.34
Aroma	**Results**	**Appearance**	**Results**	**Overall acceptance**	**Results**
Not aromatic at all	0	I did not like at all	0	I did not like at all	0
Neither aromatic nor non-aromatic	0	I do not like	0	I do not like	0
Not aromatic	0	I neither liked nor disliked	0	I neither liked nor disliked	0
Slightly aromatic	0	I liked it very little	0	I liked it very little	0
Medium aroma	53.33	I liked it a little	6.68	I liked it a little	6.68
Very aromatic	13.34	I like	46.66	I like	46.66
In perfect aroma	33.33	I like it a lot	46.66	I like it a lot	46.66
Purchase intent	**Results**				
Yes	100				
No	0				

4. Conclusion

In the present study, fish soup from anchovy with high nutritional content was prepared and the proximate composition and sensory quality parameters of the fish soup were evaluated. Based on the findings of the proximate composition analysis, it has been shown that anchovy soup has a noteworthy nutritional composition. The anchovy soup was highly appreciated sensory-wise, and all the panelists reported their desire to purchase the product. In this respect, it has been seen that anchovy soup will provide significant advantages in increasing healthy nutrition through different consumption methods for anchovy fish, which is the most important hunting product of Türkiye. Combining the consumption of anchovy with different tastes in regional cuisines will contribute to diversity. In addition, it is predicted that serving anchovy fish in the form of soup will make positive contributions to the consumption habits of people who are prejudiced against fish consumption and do not consume enough fish. Additionally, future studies to determine the amino acid and fatty acid composition of fish soup will provide more detailed information about the nutritional composition of fish soup.

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Conflict of Interest

The authors have no conflict of interest to declare.

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ARAŞTIRMA MAKALESİ | RESEARCH ARTICLE

Alabalık (*Oncorhynchus mykiss*) Yetiştiriciliğinde Sabit Su Sıcaklığında Farklı Yemleme Yüzdelerinin İç Organ Yüzdesi ve Füme Verimliliği Üzerindeki Etkisi

The Effect of Different Feeding Percentages on Internal Organ Percentage and Smoke Efficiency at Constant Water Temperature in Trout (*Oncorhynchus mykiss*) Cultivation

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Anahtar Kelimeler
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İç organ
Yemleme

ÖZET

Bu çalışmada iki farklı havuza başlangıç kilogramı aynı olan alabalıklar konularak bir grubu yemleme yüzdesi Skretting yem firmasının verdiği tabloya göre, diğer gruba doyuncaya kadar yemleme yapılmıştır. 120 gr başlangıç ağırlığı olan alabalıklar 8000'er adet olacak şekilde yerleştirilmiştir. Deneme 59 gün sürmüştür. Deneme tamamlandığında balıklar hasat edilip her iki gruptaki iç organ yüzdeleri karşılaştırılarak aradaki farklar tespit edilmiştir. Deneme sonucunda su sıcaklığına bağlı doğru yüzdeyle yemlemenin faydalı olacağı görülmüştür. Doğru yemlemenin artan üretim maliyetlerini aşağıya çekeceği tespit edilmiştir.

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ABSTRACT

In this study, trout with the same initial weight were placed in two different ponds, one group was fed according to the feeding percentage table given by the Skretting feed company, and the other group was fed until they were satisfied. 8000 trout with an initial weight of 120 grams were placed in the ponds. The trial lasted 59 days. When the experiment was completed, the fish were harvested internal organ percentages in both groups were compared and the differences were determined. As a result of the experiment, it was seen that feeding with the correct percentage depending on the water temperature would be beneficial. It was determined that correct feeding would reduce the increasing production costs.

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1. Giriş

Dünyada artan nüfusun besin ihtiyaçlarını karşılayamaması insanoğlunun besin kaynağı olarak balık üretim faaliyetlerine daha fazla yönelmelerine neden olmuştur. Alabalık üreticiliği tatlı sularda bu faaliyetlerin en önemli bölümünü oluşturmaktadır. Etin lezzetli olması ve piyasa değerinin yüksek olmasının yanı sıra alabalık yetiştiriciliğine uygun alanların çokluğu alabalık üretiminin ve yetiştiriciliğinin hızla gelişmesinin temel nedenidir. Günümüzde gelişen teknolojilerin yeni bilgilerin ışığında Türkiye'de alabalık (*Oncorhynchus mykiss*) üretimi hızla artmaktadır. TÜİK verilerine göre 2000 yılında 44533 ton alabalık üretimi yaparken 2022 yılında bu rakam 145 bin 649 ton olarak belirlenmiştir (TÜİK, 2023). Gökkuşağı alabalığı diğer türlerine göre yetiştiriciliğinin yapılması daha elverişli bir

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türdür. Bunun sebepleri arasında 9-12 ay gibi kısa sürede porsiyonluk ağırlığa (250-300 g) ulaşması, hastalıklara karşı dirençli olması ve üretiminin daha kolay olması gibi faktörler ön plana çıkmaktadır. Ayrıca aktif yem alması, yemlenmesinin kolay olması ve yem değerlendirme oranın iyi olması bakımından da tercih edilmektedir. Yaşam ortamı bakımından berrak, temiz, serin ve oksijen yönünden zengin suları tercih eden alabalık Türk halkı tarafından özellikle etinin lezzetli oluşuyla aranan balıklar arasında bulunmaktadır. Günümüz alabalık üretiminde artan yem ve işleme maliyetleri yemin çok doğru hazırlanıp balıklara doğru bir şekilde verilmesinin önemini ortaya çıkarmıştır. Ülkemizde üretilen alabalıklar iç ve dış tüketime, taze soğutulmuş, dondurulmuş ve füme olarak sunulmaktadır (Açıl & Demirci, 1984; Çelebi, 1995; Babadoğan, 1998; Aydın, 2000; Adıgüzel & Akay, 2005). Dumanlanmış (füme) alabalık, kışın yaprağını döken sert ağaçların odun talaşı ile elde edilen dumanın içerisinde belirli teknikler ile tuzlanmış ve marine edilmiş taze balıkların bekletilmesi ile oluşturulmuş, ekstra lezzetlendirilmiş, aroma kazandırılmış ve saklama süreleri arttırılmış ürünlerdir. Bu çalışma, sabit su sıcaklığında farklı yemleme koşullarında beslenen alabalıkların büyüme performansı, yemin ete dönüşüm oranı ve füme verimliliği arasındaki farklılıkları belirleyerek doğru yemlemenin önemini vurgulanmayı amaçlanmaktadır.

2. Materyal ve Yöntem

Bu çalışmada yürütülen denemeler, Muğla ili Seydikemer ilçesinde faaliyet gösteren, 48-206 su ürünleri yetiştiricilik belge numarasına sahip ve 120 ton/yıl üretim kapasiteli alabalık yetiştiriciliği tesisinde gerçekleştirilmiştir. Tesisde 31 havuz olup A kademesinde bulunan 15 ve 16 numaralı havuzlar deneme yapmak için ayrılmıştır.

Yem denemesi amacı ile ayrılan havuzlardan bir tanesinde doyuncaya kadar yemleme, bir diğerini de yemleme tablosuna bağlı kalarak balık büyüklüğü ve su sıcaklığına göre yemleme yapılmıştır. Deneme yapılacak tesisin su sıcaklığı deneme süresi boyunca 12,7-13,2 santigrat derece arasında, sudaki oksijen çözünürlükleri de girişte en yüksek 9,9 ppm çıkışta en düşük 7 ppm olarak ölçülmüştür. Satürasyon (Suyun oksijen doygunluğu) %95 civarıdır. Deneme havuzları betonarme yapılar olup dikdörtgen şeklindedir. Havuz ebatları her iki havuz için aynı olup boy 25,8 metre en 3,5 metre ve derinlik 1,1 metre olarak ölçülmüştür. Havuzların hacimleri her iki havuz için 99,3 metre küptür. Denemenin sadece yeme odaklanması diğer bileşenlerinin tamamen aynı olması için su girişleri saniyede 28 litre olarak ayarlanmıştır. Bu veriler ışığında dakikada havuzlara 1680 litre su akması planlanmıştır. Bu da saatte yaklaşık 100 ton su girişi olması demektir. Böylece 24 saatlik süreçte havuz su değişimini 24 kez olmasını sağlanmıştır. Alabalık üretimlerinde havuz su değişimi günde 20-24 olması ideal olarak kabul edilir. Yem denemesine 14.03.2023 tarihinde başlanmıştır. Her iki deneme havuzuna da ortalama ağırlıkları 120 g olan toplam 8000 adet alabalık konulmuştur. Her iki havuz yem denemesine her yönden eşit özelliklerle hazır hale getirilmiştir. Denemesi yapılacak balıkların doğum tarihi (sağım tarihi) 02.08.2022'dir. Havuz izleme formlarından izlenilebilirlik prosedüründen sağım tarihini bilinmektedir. Denemesi yapılacak yem Skretting yem firmasının Trial Product G 3PS (5 MM) yemi kullanılmıştır. Etiket bilgilerinde verilen kimyasal analiz sonuçlarına göre kullanılan yem; %43,6 ham protein, %2 ham yağ, %2,9 ham selüloz; %8 ham kül, %1,6 kalsiyum, %1,14 fosfor ve %0,3 sodyum içermektedir. Deneme sonrasında yemin hedef değer ve analiz sonuçlarını Skretting yem firmasından istemiş olup Tablo 1'de verilmiştir. Yemin üretim tarihi 19.01.2023 olup son kullanma tarihi 18.07.2023'tür. Kullanılan yemin lot numarası 0015427684 olarak kayıt altına alınmıştır.

Tablo 1. Skretting yem üretim anonim şirketi yemleme tablosu °C/g.

Ağırlık (g)	2 °C	3 °C	4 °C	5 °C	6 °C	7 °C	8 °C	9 °C	10 °C	11 °C	12 °C	13 °C	14 °C
% (Balık ağırlığının yüzdesi oranında verilmesi gereken miktar)													
0,2	1,38	1,57	1,76	1,97	2,18	2,41	2,65	2,9	3,17	3,44	3,72	4,02	4,33
0,4	1,22	1,39	1,57	1,77	1,97	2,18	2,41	2,64	2,89	3,15	3,42	3,7	3,99
0,6	1,14	1,3	1,47	1,66	1,85	2,06	2,27	2,5	2,74	2,99	3,25	3,52	3,8
0,8	1,08	1,24	1,4	1,58	1,77	1,97	2,18	2,4	2,63	2,88	3,13	3,39	3,67
1	1,03	1,19	1,35	1,53	1,71	1,91	2,11	2,33	2,55	2,79	3,04	3,3	3,57
2	0,91	1,05	1,2	1,36	1,53	1,71	1,91	2,11	2,32	2,54	2,78	3,02	3,27
3	0,83	0,97	1,11	1,27	1,43	1,61	1,79	1,99	2,19	2,41	2,63	2,87	3,11
4	0,79	0,92	1,06	1,21	1,37	1,54	1,72	1,9	2,1	2,31	2,53	2,76	3
5	0,75	0,88	1,01	1,16	1,32	1,48	1,66	1,84	2,04	2,24	2,46	2,68	2,92
6	0,72	0,85	0,98	1,12	1,28	1,44	1,61	1,79	1,98	2,18	2,4	2,62	2,85
7	0,7	0,82	0,95	1,09	1,24	1,4	1,57	1,75	1,94	2,14	2,35	2,56	2,79
8	0,68	0,8	0,93	1,07	1,21	1,37	1,54	1,71	1,9	2,1	2,3	2,52	2,74
9	0,66	0,78	0,91	1,04	1,19	1,34	1,51	1,68	1,87	2,06	2,26	2,48	2,7

Tablo 1. (devam ediyor)

Ağırlık (g)	2 °C	3 °C	4 °C	5 °C	6 °C	7 °C	8 °C	9 °C	10 °C	11 °C	12 °C	13 °C	14 °C
	% (Balık ağırlığının yüzdesi oranında verilmesi gereken miktar)												
10	0,65	0,76	0,89	1,02	1,17	1,32	1,48	1,66	1,84	2,03	2,23	2,44	2,66
20	0,55	0,66	0,77	0,9	1,03	1,17	1,32	1,48	1,65	1,83	2,02	2,22	2,42
30	0,5	0,6	0,71	0,83	0,95	1,09	1,23	1,39	1,55	1,72	1,9	2,09	2,29
40	0,47	0,56	0,66	0,78	0,9	1,03	1,17	1,32	1,48	1,64	1,82	2	2,19
50	0,44	0,53	0,63	0,74	0,86	0,99	1,12	1,27	1,42	1,58	1,75	1,93	2,12
60	0,42	0,51	0,61	0,71	0,83	0,95	1,08	1,23	1,38	1,54	1,7	1,88	2,06
70	0,4	0,49	0,58	0,69	0,8	0,92	1,05	1,19	1,34	1,5	1,66	1,83	2,02
80	0,39	0,47	0,56	0,67	0,78	0,9	1,03	1,16	1,31	1,46	1,62	1,79	1,97
90	0,37	0,46	0,55	0,65	0,76	0,88	1	1,14	1,28	1,43	1,59	1,76	1,93
100	0,36	0,44	0,53	0,63	0,74	0,86	0,98	1,11	1,25	1,4	1,56	1,73	1,9
110	0,35	0,43	0,52	0,62	0,72	0,84	0,96	1,09	1,23	1,38	1,53	1,7	1,87
120	0,34	0,42	0,51	0,61	0,71	0,82	0,94	1,07	1,21	1,35	1,51	1,67	1,84
130	0,33	0,41	0,5	0,59	0,7	0,81	0,93	1,05	1,19	1,33	1,49	1,65	1,82
140	0,33	0,4	0,49	0,58	0,68	0,79	0,91	1,04	1,17	1,31	1,47	1,62	1,79
150	0,32	0,39	0,48	0,57	0,67	0,78	0,9	1,02	1,16	1,3	1,45	1,6	1,77
200	0,29	0,36	0,44	0,53	0,62	0,73	0,84	0,96	1,08	1,22	1,36	1,51	1,67
250	0,27	0,33	0,41	0,49	0,59	0,68	0,79	0,91	1,03	1,16	1,3	1,44	1,59
300	0,25	0,31	0,39	0,47	0,55	0,65	0,75	0,86	0,98	1,11	1,24	1,38	1,53
350	0,23	0,3	0,37	0,44	0,53	0,62	0,72	0,83	0,94	1,06	1,19	1,33	1,47

Skretting yem firması 5 mm yem kullanarak daha önce deneme yapmış ve bu deneme sonucunda elde edilen veriler ışığında çalışmanın yem programını planlamıştır. Yemleme programında balığın ağırlığına ve su sıcaklığına bağlı verilecek yem oranı tespit edilmektedir. Yapılan denemede başlangıçta 120 gram olan balığa 13 °C de %1,67 oranında yem verilmesi gerektiği bilinmektedir. Diğer bir tespit şudur: sabit su sıcaklığında balığın gramajı arttıkça günlük alacağı yem yüzdesi azalmaktadır.

Skretting yem firmasının Planladığı yemleme tablosu doğrultusunda A15 numaralı havuz yani kontrollü yemleme yapılacak olan havuza 1. günden 59. güne kadar balığa verilecek yem miktarlarını, günlük olarak balığın ağırlığındaki artış miktarı ve oranı, günlük FCR oranını ve balığın adet ve havuzdaki toplam balık ağırlığını yani biomasın artış miktarlarını ve artış oranları tahmin edilen tablo deneme başlamadan önce hazırlanmıştır (Tablo 2). Örneğin 1. gün havuzdaki toplam balığın ağırlığı 960 kg'dır. Skretting yem firmasının verdiği yemleme yüzdesindeki miktar %1,67'dir. 960 kg'ın %1,67'si 16 kilogramdır. Balığa verilmesi gereken yem miktarı 16 kg'dır. Bu doğrultuda 1. gün sonunda kazanılması gereken ağırlık 17,8 kg'dır. Tahmini FCR (balığa verilen yem/balığın kazandığı ağırlık) 0,91'dir. 2. gün havuzdaki balıkların ağırlığı 977,8 kg olması beklenmiştir. Balık adet bazında 1. gün 120 gram olan balık 2. gün 122,2 gram olması tahmin edilmiştir. Balığa verilmesi gereken yemleme yüzdesi oranları Skretting yem firmasından temin edilmiştir. Denemeye başlandığındaki alabalığın ilk ağırlığı 120 gramdır. Deneme yapılan havuzların oksijen ve sıcaklık değer tablosu Tablo 3'de verilmiştir.

Tablo 2. Kontrollü yemleme grubunun hesaplama tablosu.

Gün	Havuz tahmini kg	Havuz adet	Yemleme yüzdesi	Tahmini FCR	Atılması gereken yem	Günlük kazanılan ağırlık	Günlük tahmini ortalama
1	960,0	8000	1,67	0,91	16,0	17,8	120,0
2	977,8	8000	1,67	0,91	16,3	18,1	122,2
3	996,0	8000	1,67	0,91	16,6	18,5	124,5
4	1014,4	8000	1,67	0,91	16,9	18,8	126,8
5	1033,3	8000	1,67	0,91	17,3	19,2	129,2
6	1052,4	8000	1,65	0,91	17,4	19,3	131,6
7	1071,7	8000	1,65	0,91	17,7	19,6	134,0
8	1091,4	8000	1,65	0,91	18,0	20,0	136,4

Tablo 2. (devam ediyor)

Gün	Havuz tahmini kg	Havuz adet	Yemleme yüzdesi	Tahmini FCR	Atılması gereken yem	Günlük kazanılan ağırlık	Günlük tahmini ortalama
9	1111,4	8000	1,65	0,91	18,3	20,4	138,9
10	1131,8	8000	1,65	0,91	18,7	20,7	141,5
11	1152,5	8000	1,65	0,91	19,0	21,1	144,1
12	1173,6	8000	1,65	0,91	19,4	21,5	146,7
13	1195,2	8000	1,65	0,91	19,7	21,9	149,4
14	1217,1	8000	1,65	0,91	20,1	22,3	152,1
15	1239,4	8000	1,61	0,91	20,0	22,2	154,9
16	1261,6	8000	1,61	0,91	20,3	22,6	157,7
17	1284,1	8000	1,61	0,91	20,7	23,0	160,5
18	1307,1	8000	1,61	0,91	21,0	23,4	163,4
19	1330,5	8000	1,61	0,91	21,4	23,8	166,3
20	1354,3	8000	1,61	0,91	21,8	24,2	169,3
21	1378,5	8000	1,61	0,91	22,2	24,7	172,3
22	1403,2	8000	1,61	0,91	22,6	25,1	175,4
23	1428,3	8000	1,61	0,91	23,0	25,5	178,5
24	1543,8	8000	1,61	0,91	23,4	26,0	181,7
25	1479,8	8000	1,61	0,91	23,8	26,5	185,0
26	1506,3	8000	1,61	0,91	24,3	26,9	188,3
27	1533,2	8000	1,61	0,91	24,7	27,4	191,7
28	1560,7	8000	1,61	0,91	25,1	27,9	195,1
29	1588,6	8000	1,61	0,91	25,6	28,4	198,6
30	1617,0	8000	1,51	0,91	24,4	27,1	202,1
31	1644,1	8000	1,51	0,91	24,8	27,6	205,5
32	1671,7	8000	1,51	0,91	25,2	28,0	209,0
33	1699,8	8000	1,51	0,91	25,7	28,5	212,5
34	1728,3	8000	1,51	0,91	26,1	29,0	216,0
35	1757,3	8000	1,51	0,91	26,5	29,5	219,7
36	1786,8	8000	1,51	0,91	27,0	30,0	223,3
37	1816,7	8000	1,51	0,91	27,4	30,5	227,1
38	1847,2	8000	1,51	0,91	27,9	31,0	230,9
39	1878,2	8000	1,51	0,91	28,4	31,5	234,8
40	1909,7	8000	1,51	0,91	28,8	32,0	238,7
41	1941,8	8000	1,51	0,91	29,3	32,6	242,7
42	1974,3	8000	1,51	0,91	29,8	33,1	246,8
43	2007,5	8000	1,51	0,91	30,3	33,7	250,9
44	2041,1	8000	1,44	0,91	29,4	32,7	255,1
45	2073,8	8000	1,44	0,91	29,9	33,2	259,2
46	2107,0	8000	1,44	0,91	30,3	33,7	263,4
47	2140,7	8000	1,44	0,91	30,8	34,3	267,6
48	2175,0	8000	1,44	0,91	31,3	34,8	271,9
49	2209,8	8000	1,44	0,91	31,8	35,4	276,2
50	2245,1	8000	1,44	0,91	32,3	35,9	280,6
51	2281,0	8000	1,44	0,91	32,8	36,5	285,1
52	2317,5	8000	1,44	0,91	33,4	37,1	289,7
53	2354,6	8000	1,44	0,91	33,9	37,7	294,3
54	2392,3	8000	1,44	0,91	34,4	38,3	299,0
55	2430,6	8000	1,44	0,91	35,0	38,9	303,8
56	2469,4	8000	1,44	0,91	35,6	39,5	308,7
57	2509,0	8000	1,44	0,91	36,1	40,1	313,6
58	2549,1	8000	1,44	0,91	36,7	40,8	318,6
59	2589,9	8000	1,44	0,91	37,3	41,4	323,7

Tablo 3. Deneme yapılan havuzların oksijen ve sıcaklık değer tablosu.

		A15	A15	A15	A15	A16	A16	A16	A16
	Su sıcaklığı (°C)	**Su giriş oksijen (ppm)**	**Oksijen doygunluğu (%)**	**Su çıkış oksijen (ppm)**	**Oksijen doygunluğu (%)**	**Su giriş oksijen (ppm)**	**Oksijen doygunluğu (%)**	**Su çıkış oksijen (ppm)**	**Oksijen doygunluğu (%)**
14.03.2023	12,7	9,8	99	7,7	85	9,6	91	7,7	85
15.03.2023	12,7	9,9	100	7,8	89	9,9	99	8	81
16.03.2023	12,7	9,9	99	7,8	87	9,8	99	8	79
17.03.2023	12,7	9,9	97	7,9	85	9,7	89	8	86
18.03.2023	12,7	9,6	97	7,9	91	9,6	97	7,9	91
19.03.2023	12,7	9,8	99	8	89	9,9	99	7,9	85
20.03.2023	12,7	9,8	105	8	89	9,8	99	7,6	87
21.03.2023	12,7	9,9	104	7,9	91	9,8	99	8	87
22.03.2023	12,7	9,7	103	7,7	91	9,8	99	7,8	89
23.03.2023	12,7	9,8	99	7,6	87	9,6	91	7,7	85
24.03.2023	12,8	9,5	99	8	85	9,8	91	7,1	92
25.03.2023	12,8	9,6	99	8	97	9,9	91	7,1	92
26.03.2023	12,8	9,8	99	7,6	87	9,6	91	7,4	91
27.03.2023	12,8	9,4	102	7,9	85	9,8	97	7,4	91
28.03.2023	12,8	9,5	103	8	82	9,8	94	7,5	92
29.03.2023	12,8	9,6	97	7,9	91	9,7	94	7,5	91
30.03.2023	12,8	9,6	99	7,7	86	9,6	91	7,7	85
31.03.2023	12,8	9,7	100	7,8	87	9,7	97	7,6	92
1.04.2023	12,8	9,6	91	7,7	85	9,8	97	7,5	91
2.04.2023	12,8	9,8	102	8	87	9,8	94	7,4	91
3.04.2023	12,8	9,9	99	7,9	89	9,6	97	7,9	91
4.04.2023	12,8	9,7	99	7,7	91	9,8	97	7,5	91
5.04.2023	12,8	9,8	102	7,8	91	9,9	97	7,5	91
6.04.2023	12,9	9,6	91	7,7	85	9,9	97	7,6	89
7.04.2023	12,9	9,6	99	7	92	9,6	97	7,9	91
8.04.2023	12,9	9,8	99	8	92	9,8	99	7,7	91
9.04.2023	12,9	9,8	99	7,9	89	9,6	91	7,7	85
10.04.2023	12,9	9,6	99	8	89	9,7	97	7,8	89
11.04.2023	12,9	9,9	102	8	87	9,8	97	7,8	89
12.04.2023	12,9	9,9	102	8	89	9,8	91	7,8	91
13.04.2023	12,9	9,7	97	7,7	89	9,5	95	7,7	87
14.04.2023	12,9	9,7	95	7,5	89	9,5	95	7,7	87
15.04.2023	12,9	9,8	96	7,5	90	9,6	95	7,8	88
16.04.2023	12,9	9,8	95	7,8	90	9,6	96	7,8	88
17.04.2023	12,9	9,6	94	7,8	90	9,6	96	7,8	87
18.04.2023	12,9	9,6	94	7,8	88	9,6	96	8	87
19.04.2023	12,9	9,6	94	7,8	88	9,6	95	8	85
20.04.2023	12,9	9,5	95	8	89	9,5	95	8	85
21.04.2023	12,9	9,5	96	8	87	9,5	94	8	85
22.04.2023	12,9	9,3	102	8	87	9,5	95	8,1	85
23.04.2023	13	9,3	93	8	87	9,6	95	8,1	85
24.04.2023	13	9,1	93	7,8	89	9,6	95	8,1	81
25.04.2023	13	9,3	89	7,8	89	9,6	96	7,7	81
26.04.2023	13	9,3	89	7	89	9,7	96	7,7	81
27.04.2023	13	9,4	89	8	87	9,7	95	7,8	81
28.04.2023	13	9,4	89	8	87	9,7	95	7,8	83
29.04.2023	13	9,4	88	8	87	9,7	95	7,8	83
30.04.2023	13	9,5	97	8	89	9,8	97	7,8	83
1.05.2023	13	9,5	97	7,8	89	9,8	95	8	85
2.05.2023	13,1	9,6	97	8,1	88	9,5	95	8	85
3.05.2023	13,1	9,6	95	8	87	9,5	95	8	84
4.05.2023	13,1	9,5	96	8	90	9,6	95	8	84
5.05.2023	13,2	9,4	96	8	90	9,6	98	7,8	84
6.05.2023	13,2	9,4	95	8,4	90	9,6	97	7,8	85
7.05.2023	13,2	9,6	94	8,1	89	9,6	95	7	85
8.05.2023	13,2	9,6	95	8,1	89	9,6	95	7,1	85
9.05.2023	13,2	9,6	96	8,1	89	9,6	96	7,1	84
10.05.2023	13,2	9,4	96	7,9	89	9,6	96	7,5	84
11.05.2023	13,2	9,4	96	7,9	89	9,6	95	7,6	84
12.05.2023	13,2	9,6	97	7,9	88	9,4	95	7,6	85

Deneme yapılan alabalık üretim tesisinin deneme yapıldığı süre boyunca su sıcaklığı 12,7-13,2 °C arasındadır ve büyük bir değişim saptanmamıştır. Bu nedenle deneme boyunca su sıcaklığı 13 °C olarak kabul edilmiştir. Havuzların su girişlerindeki oksijen değerleri ve oksijen doygunluk seviyeleri havuzun çıkışına göre daha yüksek değerdedir ve bu rakamlarda da olağandışı değişim tespit edilmemiştir. 50 gr'dan daha büyük balıkların toplam 1 kg'nın 1 saatte 400-500 mg oksijen tükettikleri bilinmektedir. Ayrıca kullanılan suyun havuzlardan çıkışta litrede 6 mg oksijen içermesi zorunluluktur.

3. Bulgular

Denemeye başlandığı ilk günden itibaren her gün havuza atılan yem miktarlarını ve balığın ağırlığındaki artış miktarları kayıt altına alınmıştır. Havuzdan çıkan ölü balıkların adet ve ağırlıkları da kayıt altına alınmıştır. Kontrollü yemleme yapılan havuzda balığa verilen yem miktarlarında düzenli bir artış göstermiştir. Doyuncaya kadar yemleme yapılan havuzda ise günlük verilen yem miktarlarında düzensizlik olduğu gözlemlenmiştir. Bir gün fazla yem yiyen balık ertesi gün yediği yem oranı düşük çıkmıştır. Bir sonraki gün ise artış olduğu gözlemlenmiştir (Tablo 4). Deneme sonunda alabalık ağırlığı 323 gramdır.

Tablo 4. Deneme havuz takip tablosu.

		A15	A16	A15	A15	A16	A16
Gün	**Tarih**	**Yem G**	**Yem G**	**Ölü adet**	**Ölü gr**	**Ölü adet**	**Ölü gr**
1	14.03.2023	16,0	20,2	0	0	0	0
2	15.03.2023	16,3	19,3	0	0	2	214
3	16.03.2023	16,6	11,1	0	0	0	0
4	17.03.2023	16,9	24,2	0	0	0	0
5	18.03.2023	17,3	17,7	0	0	0	0
6	19.03.2023	17,4	22,8	1	162	0	0
7	20.03.2023	17,7	14,3	0	0	0	0
8	21.03.2023	18,0	21,6	0	0	0	0
9	22.03.2023	18,3	19,6	0	0	1	176
10	23.03.2023	18,7	13,8	0	0	0	0
11	24.03.2023	19,0	15,9	2	410	0	0
12	25.03.2023	19,4	23,6	0	0	0	0
13	26.03.2023	19,7	17,8	0	0	0	0
14	27.03.2023	20,1	20,6	0	0	0	0
15	28.03.2023	20,0	17,3	0	0	0	0
16	29.03.2023	20,3	18,5	0	0	0	0
17	30.03.2023	20,7	23,8	0	0	0	0
18	31.03.2023	21,0	16,9	1	236	0	0
19	1.04.2023	21,4	15,7	0	0	0	0
20	2.04.2023	21,8	28,6	0	0	1	220
21	3.04.2023	22,2	17,2	0	0	0	0
22	4.04.2023	22,6	22,5	0	0	0	0
23	5.04.2023	23,0	25,6	1	240	1	200
24	6.04.2023	23,4	19,1	0	0	0	0
25	7.04.2023	23,8	26,8	0	0	0	0
26	8.04.2023	24,3	27,9	0	0	0	0
27	9.04.2023	24,7	21,1	0	0	0	0
28	10.04.2023	25,1	23,8	2	448	0	0
29	11.04.2023	25,6	24,7	0	0	0	0
30	12.04.2023	24,4	29,9	0	0	1	260
31	13.04.2023	24,8	17,4	0	0	0	0
32	14.04.2023	25,2	24,6	0	0	0	0
33	15.04.2023	25,7	25	0	0	0	0
34	16.04.2023	26,1	23,6	0	0	0	0
35	17.04.2023	26,5	24,2	0	0	0	0
36	18.04.2023	27,0	30,4	0	0	0	0
37	19.04.2023	27,4	31	0	0	0	0

Tablo 4. (devam ediyor)

		A15	A16	A15	A15	A16	A16
Gün	**Tarih**	**Yem G**	**Yem G**	**Ölü adet**	**Ölü gr**	**Ölü adet**	**Ölü gr**
38	20.04.2023	27,9	22,7	0	0	0	0
39	21.04.2023	28,4	26	1	240	0	0
40	22.04.2023	28,8	33	0	0	0	0
41	23.04.2023	29,3	27,3	0	0	0	0
42	24.04.2023	29,8	30,1	0	0	0	0
43	25.04.2023	30,3	28,9	0	0	1	274
44	26.04.2023	29,4	30,7	0	0	0	0
45	27.04.2023	29,9	26,9	0	0	0	0
46	28.04.2023	30,3	34,1	0	0	0	0
47	29.04.2023	30,8	27,3	0	0	0	0
48	30.04.2023	31,3	36,3	0	0	0	0
49	1.05.2023	31,8	34,2	0	0	0	0
50	2.05.2023	32,3	36,1	1	268	1	280
51	3.05.2023	32,8	27,8	0	0	0	0
52	4.05.2023	33,4	26,9	0	0	0	0
53	5.05.2023	33,9	32,4	0	0	0	0
54	6.05.2023	34,4	35,5	0	0	0	0
55	7.05.2023	35,0	35	1	296	0	0
56	8.05.2023	35,6	31,4	0	0	1	288
57	9.05.2023	36,1	36,6	0	0	0	0
58	10.05.2023	36,7	35,9	0	0	0	0
59	11.05.2023	37,3	38	0	0	0	0

Tablo 5. Sonuç raporu.

	Kontrollü yemleme	**Doyuncaya kadar yemleme**
12,7 - 13,2 °C	**A15**	**A16**
Başlangıç adet	8000	8000
Başlangıç kg	960,00	960,00
Başlangıç orta.	120,00	120,00
Verilen yem kg	1504,2	1491,2
Final adet	7989	7991
Final kg	2630,6	2585,1
Final ortalama gr	329,28	323,50
Ölü adet	10	9
Ölü kg	2,3	1,912
Kazanılan ağırlık	1670,60	1625,10
Tanımsız adet	1	0
Uygulama süresi gün	59	59
Gramaj artışı	209,28	203,50
Günlük ortalama aldığı gram	3,55	3,45
Toplam zayiat oranı %	0,13	0,11
FCR	0,899	0,917
Başlangıç tarihi	14.03.2023	
Bitiş tarihi	11.05.2023	

59 gün boyunca ortalama 13 °C su sıcaklığında gerçekleştirilen deneme süresince tüm şartlar eşit hale getirilmiştir. Bu kapsamda her iki havuz için de havuzun su girişleri, su girişlerinde havuza akan su miktarları, havuzun büyüklüğü, havuza atılan balık adetleri (8000) ve balık ağırlıkları (960 kg) aynı ayarlanmıştır. Bir balığın başlangıç ağırlığı 120 gramdan 323 ve 329 grama yükselmiştir. Kontrollü yemleme yapılan A15 numaralı havuza deneme boyunca 1504,2 kg yem verilmiştir. Finalde havuzdaki balık adet sayısı 7989'dur. 10 adet balık ölmüştür. Toplam balık adedinde 1 adet tanımsız fark (belirlenemeyen) oluşmuştur. Bu yüzden 1 adet balık tanımsız olarak rapor edilmiştir. 10 adet ölü balığın ağırlığı 2,3 kg'dır. Başlangıçta toplam ağırlığı 960 kg olan balıkların finaldeki toplam ağırlığı 2630,6 kg'a yükselmiştir. Kazanılan ağırlık 1670,60 kg'dır. Hasat edilmeden önce 1 adet balığın ağırlığı 329,28 gram olarak tespit edilmiştir. Bir adet balıkta 209,28 gramaj artışı gözlenmiştir. Günlük ortalama aldığı gram 3,55'dir. Toplam fire oranı %0,13'dür. FCR oranı 0,899'dur. Balığa 0,899 kg yem verilmiş olup 1 kg et elde edilmiştir (Tablo 5).

Doyuncaya kadar yemleme yapılan A16 numaralı havuza deneme boyunca 1491,2 kg yem verilmiştir. Sanılanın aksine doyuncaya kadar yemleme de kontrollü yemleme kadar yem verilememiştir. Finalde havuzdaki balık adet sayısı 7991'dir. 9 adet balık ölmüştür. 9 adet ölü balığın ağırlığı 1,912 kg'dır. Başlangıçta toplam ağırlığı 960 kg olan balıkların finaldeki toplam ağırlığı 2585,1 kg'a yükselmiştir. Kazanılan ağırlık 1625,10 kg'dır. Hasat edilmeden önce 1 adet balığın ağırlığı 323,50 gram olmuştur. 1 adet balıkta 203,50 gramaj artışı gözlenmiştir. Günlük ortalama aldığı gram 3,45'dir. Toplam fire oranı %0,11'dir. FCR oranı 0,917'dir. Balığa 0,917 kg yem verilmiş olup 1 kg et elde edilmiştir. 12.05.2023 tarihinde deneme bitmiştir. Hasat etmeye 72 saat kala balıklara yem verilme kesilip balıklar aç bırakılmıştır ve 15.05.2023 tarihinde balıklar hasat edilerek işleme fabrikasına gönderilmiştir (Şekil 1).

Şekil 1. Deneme başlangıç ve bitişindeki her 2 balığın yan yana görünüşü.

4. Tartışma ve Sonuç

Denemede kontrollü yemleme ile yapılan grubun FCR'ı 0,899, kontrolsüz yemlenen grubun FCR'ı ise 0,917 olarak tespit edilmiştir. Bu sonuç kontrollü grubun kontrolsüz gruba göre yemin ete dönüşüm oranında %2'lik bir iyileştirme gerçekleştirdiğini göstermiştir. Flores vd. (2023) de alabalıkları üç ticari yem ile besleyerek gökkuşağının ticari performans ve organ gelişimi üzerindeki etkisini belirlemişlerdir. Araştırma sonucunda diyetin yem dönüşümünü ve pigmentasyonu iyileştirdiği, ticari yemlerin ise üretken performansı, pigmentasyonu ve organların göreceli ağırlığını etkilediği, ancak karkas ve fileto verimini etkilemediği sonucuna varılmıştır. Yem içeriğinin işlevselliğinin değerlendirilmesi, modern balık yemi üretim uygulamalarında hayati bir rol oynamaktadır. Draganovic vd. (2011) balık unu, buğday gluteni, soya proteini konsantreli ekstrüder yeminin etkisini sistem parametrelerini dikkate alarak balık kalitesini değerlendirmişlerdir. Sonuçlardan, bitki kökenli balık ununun değerlendirilen bitkisel protein kaynaklarında doğal olarak bulunmayan benzersiz fonksiyonel özelliklere sahip olduğu sonucuna varılmıştır.

Sonuç olarak 500 ton/yıl yem kullanan bir alabalık üretim çiftliği kontrollü yemleme ile 10 tonluk bir yem tasarrufu sağlayabilecektir. Alabalık üretim tesislerindeki artan yem maliyetleri düşünüldüğünde bu rakam küçümsenmeyecek boyuttadır. Günümüzde ortalama 1 kg yem fiyatının 2 $ olduğu düşünülürse bu rakam bir alabalık üretim tesisi için ciddi bir kazanç sağlayacaktır.

İşleme fabrikasından alınan veriler ışığında kontrollü grubun vücut ağırlığından çıkan iç organ yüzdesi 18,4'tür. Kontrolsüz grubun vücut ağırlığından çıkan iç organ yüzdesi 18,6 olarak hesaplanmıştır. Bu da kontrollü grubun kontrolsüz

gruba göre % 1,08 daha az iç organ çıkardığı ve daha fazla ete sahip olduğunu göstermiştir. Aynı zamanda 59 günlük süreçte bu sonuçlar elde edildiğine göre daha uzun üretim periyotlarında yeni çalışmaların yapılmasının da gerekliliğini ortaya koymuştur. Daha uzun süreli çalışmalarda bu rakamların olumlu yönde etkilenebileceği düşünülmektedir.

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REVIEW ARTICLE

Processing and Nutritional Quality of Sea Snail (*Rapana venosa* Valenciennes, 1846) Meat

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ABSTRACT

Sea snail, which is considered an invasive species in the Black Sea, has been caught by fishermen who earn their living in this area since the second half of the 1980s, as an alternative to other products, processed in factories and exported to countries with demand abroad. The fishing of sea snails, a highly sought-after delicacy in the Far East, has been limited due to declining stocks in the Sea of Japan caused by overfishing. Consequently, this has created export opportunities for several countries, including Turkey, and its significance has been steadily growing with the increasing export volumes. Due to their low cholesterol and fat content, and significant levels of protein and minerals, snails are among the healthiest foods consumed by humans. The fact that shellfish are sensitive and perishable products increases the sensitivity of the issue on issues such as the processing method, duration, and cold storage of these products. Therefore, ensuring that the quality reaches consumers without deterioration and monitoring it is of great importance in the export of shellfish. It will be possible for countries to reach a higher potential in snail exports and increase their market share in snail trade by obtaining products of appropriate quality. For this, it is necessary to improve the collection conditions in snail hunting, to monitor its transportation under appropriate conditions and to improve the processing stages. The purpose of the article was to provide an overview of the processing and quality of sea snail (*Rapana venosa*) meat.

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1. Introduction

The native habitat of *Rapana venosa* Valenciennes 1846 (Neogastropoda, Muricidae), often known as sea snails, includes the Sea of Japan, the Yellow Sea, the Bohai Sea, and the areas extending from the East China Sea to Taiwan (Bayraklı et al., 2016). The dissemination occurred globally by maritime vessels during World War II or, more plausibly, through the ballast water of ships when the eggs were in their larval phase. The first place detected in the Black Sea was reported by edde) in Novorossiysky Bay in 1946 (Gönener & Özsandıkçı, 2017). The sea snails, introduced to the Black Sea by ships, quickly established themselves in their new environment. Despite being non-invasive, they rapidly proliferated and were visible throughout the shores of the Caucasus, Crimea, and the Sea of Azov within a decade. Between 1959 and 1972, *Rapana thomasiana* penetrated the northwestern part of the Black Sea and reached the waters of Romania, Bulgaria and Turkey, where it proceeded to reproduce and increase in numbers. The proliferation of sea snails along the entire Black Sea coast and the southern part of the Sea of Azov occurred in only 25 years (Zolotarev, 1996). Since 1984, *Rapana venosa* has been seen migrating from the Black Sea to the Aegean and Mediterranean Seas, subsequently expanding its distribution to other regions, such as France, England, the North Sea, and the German coast (Gönener & Özsandıkçı, 2017).

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The economic significance of sea snails grew as a source of income for small-scale fishermen in the Black Sea area beginning in the 1980s (Sağlam & Düzgüneş, 2016). Fishing communities in the Black Sea have been harvesting sea snails - an invasive species- since the 1980s as a cheap substitute for other products, with the intention of processing and exporting them to areas where they are in high demand. Turkey is one of several countries that have found opportunities to export sea snails, which are highly valued in the Far East but have been severely limited in hunting due to overfishing in the Sea of Japan. As a result, sea snails are becoming increasingly important as exports continue to rise (Meraklı, 2018). Exports of sea snails reached $14,419,013 from January to September 2023, up 67% from $8,627,019 in the same period the previous year. The amount of products exported increased from 1,136 metric tons to 1,326 metric tons (Yıldız, 2023).

The habitats of the sea snail are sandy, muddy, algae environments, and around mussel beds up to a depth of 90 m. The lack of a natural predator in the Black Sea has allowed the fast expansion of a carnivorous sea snail, which is often considered the most active predator of mussels and oysters (Sağlam, 2007). An important factor contributing to the survival of this species is its ability to adjust to significant fluctuations in both salinity and temperature (Kos'yan, 2013). *Rapana venosa* has a high tolerance to varying salinity levels, ranging from 15 to 32‰ (Pirkova, 2020).

Snail meat has been eaten worldwide since ancient times, however it is not often consumed in Turkey like red meat, white meat, and fish (Olgunoğlu & Olgunoğlu, 2008). According to Gökhan and Sağlam (2009), these meals are considered luxury items since they are sourced from nature and exported abroad. Sea snails, which have gained significance as a valuable export commodity in our country, are marketed in several forms including live, fresh meat, frozen, cooked frozen, canned, and pickled. Various preparations of sea snail are more highly regarded in culinary traditions. According to Sürer (2013), it is often enjoyed in North American salads and soups, while in the Far East it is more commonly eaten raw or in canned form.

Sea snails, or "ashtrays," are an important source of revenue in the Black Sea area and the nation as a whole due to their widespread capture, processing, and export. The *Rapana venosa* that is captured in the Eastern Black Sea Region is packed into 50-60 kilogram sacks and transported to processing companies by trucks. Once there, it undergoes a series of processes that are necessary for export (Arslan, 2009).

The manufacturing process for the sea snail involves many steps after the quality check of the raw material obtained via hunting. These steps include steam cooking at a temperature of 100 °C for 10 minutes, separating the shell, removing the internal organs, chilling the snail at a temperature of 4 °C, and washing it thoroughly. During the calibration process, sea snails are measured and categorized based on their size. They are then allowed to rest before being filled and weighed. After that, they are frozen and packed at a temperature of -45°C. Finally, they are kept for future use (İrkin et al., 2007).

Preserving food for a healthy life has grown more important as society's eating habits have evolved from the past to the present, especially with the rise of ready-to-eat meals (Karslı, 2013). According to Özgür (2005), one-third of the protein needed for a balanced diet each day must come from animal sources, and the significance of a healthier and more balanced diet is growing as people get more understanding about the topic. Because it provides for a large portion of people's nutritional requirements, aquaculture is seen as a possible response to the world's growing population and food require.

In addition to its economic importance, snails are a valuable food in terms of nutritional content, as they are rich in mineral salts, copper, zinc, calcium and phosphorus. Snails are beneficial not only for food consumption but also for the treatment of some diseases in the medical fiel (Sağlam et al., 2003). A study on hemocyanin, a protein in invertebrate blood that transports oxygen, discovered that hemocyanins from the snails *Rapana venosa* (RvH) and *Helix vulgaris* (HvH) had specific immuno-adjuvant properties that could be activated by cell-mediated immunity. Guerin acid showed increased resistance to tumor progression in tumor-bearing animals treated with RvH and HvH compared to non-immunized animals and showed a significant immune activation, much higher than that in the Keyhole limpet haemocyanin (KLH)-immunized control group. It showed the highest survival rate in animals treated with HvH, RvH, and KLH compared to unimmunized animals (Iliev et al., 2008).

2. Systematics of the Sea Snail (*Rapana venosa*)

Sea snail, whose Latin name is *Rapana venosa*, belongs to the Muricidae family of the Mollusca (molluscs) phylum and Gastropoda (gastropod) class in the scientific classification (Kıran, 2015) (Figure 1).

Figure 1. *Rapana venosa*.

Regnum: *Animalia*

Phylum: *Mollusca*

Class: *Gastropoda*

Subclass: *Prosobranchi*

Family: *Muricidae*

Genus: *Rapana*

Species: *Rapana venosa* Valenciennes, 1846

3. Frozen Sea Snail (*Rapana venosa*) Meat Processing and Nutritional Quality

3.1. Frozen Sea Snail (*Rapana venosa*) Meat Processing

Sea snails captured in the Black Sea Region and transported to the sea snail processing plant in perforated sacks undergo sensory evaluation at the first step of raw material acceptance, and then samples are taken from the processing stages.

The workflow of the Sea Snail Meat Processing Plant, as described by Korkmaz and Pinal (2022), is shown in Figure 2.

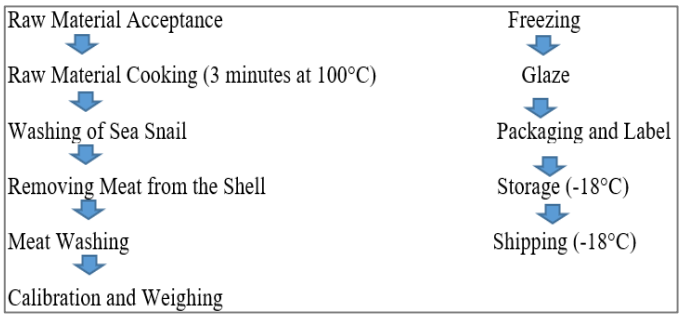

Figure 2. Sea snail processing plant workflow (Korkmaz & Pinal, 2022).

3.1.1. Raw material acceptance

Sea snails are transported to the seafood processing plant using a refrigerated transport vehicle that is stacked to provide for proper air circulation. If the microbial load in the raw material grows along with the rise in temperature over time, resulting in deformations, bruises, and deceased organisms in the sea snail flesh, these abnormalities do not meet the requirements for accepting the raw material. Once assigned a lot number based on their arrival order and weighed, the live sea snails are carefully kept away from any contact with water until they undergo processing. (Figure 3).

Figure 3. The sea snails in perforated sacks.

3.1.2. Raw material cooking

After the product raw material is entered, the cooking process is started. The live snails that have started the cooking process are thrown into the boiling water after the water in the cooking pots boils, and the cooking process is completed in 3 minutes after the water starts to boil again. The baking process is above 100°C (Figure 4.).

Figure 4. Boiling of sea snails.

3.1.3. Washing of sea snails

The output of the cooking boiler is sent to the shell washing machine for water-based cleaning. The sanitized product is put in sterilized plastic containers to purify the water. The water has been fully depleted inside plastic boxes. In this process, the cleaned product is conveyed to the pre-cooling area using plastic containers (Figure 5.).

Figure 5. Washing of sea snails in the washing drum.

3.1.4. Pre-cooling

The product undergoes pre-cooling to a temperature of 0 °C after the washing process. Consequently, the product is stored in a regulated environment to preserve its quality before processing.

3.1.5. Removing meat from the shell

Sea snails are swiftly handled with sharp equipment such as scissors and forks once they get to the product processing benches of the plant. The guts of the snails that are delivered to the booths are cleaned with scissors after they have been pulled off of their shells using a fork (Figure 6).

Figure 6. The process of separating snails from shells and entrails.

3.1.6. Washing of meat

The meat is transferred from plastic crates to washing tanks, where it is cleaned using facility water that satisfies the standards for drinking water. After the washing process is completed, the meat is placed in disinfected plastic cases (Figure 7).

Figure 7. Washing of sea snails meat in pan.

3.1.7. Selecting

The employees start picking out the products from the sanitized plastic containers. The ones that have spoiled and changed color are sorted (Figure 8).

Figure 8. Selecting of meats in bands.

3.1.8. Grading

Following the process of washing, the sea snails are transferred into plastic containers and then inserted into the grading machine. The washed and graded snail meats are then sorted into disinfected plastic cases based on their size (Figure 9).

Figure 9. Grading of sea snail meats.

3.1.9. Weighing and freezing

Following the process of washing and calibrating, the snail meat is weighed in crates and stored in the freezer's shock chamber to undergo freezing prior to packaging. The snail meat is subjected to freezing temperatures in a freezing chamber, specifically ranging between -35 °C and -40 °C. During this process, the internal temperature of the meat reaches -18 °C.

Once taken out of the freezer, the snail meat is coated in a glaze. Glazing involves coating the product's surface with water to prevent ice burns and protect it from the freezing process that follows. The sea snail meat is then packed into polyethylene bags and then into cardboard boxes using blocks. Prior to shipping, the packed and frozen products are kept at a temperature of -18 °C.

3.2. Nutritional Quality of Sea Snail (*Rapana venosa*) Meats

Sea snails are caught, processed and exported to Japan and some European countries. In terms of nutritional value, sea snails, with an average protein content of 12.95% and phosphorus content of 0.65 mg/kg, are a valuable product for healthy nutrition (İrkin et al., 2007). Table 1 shows that snail meat is an essential part of a healthy diet, even if its protein level is lower than that of red meat and much higher than that of milk. According to Kocabaş and Fenercioğlu (1992), snail meat is an excellent alternative for other meats due to its high protein and low fat content.

Table 1. The ratios of dry matter, protein, and lipids in snail meat compared to other foods (%) (Kocabaş & Fenercioğlu, 1992).

Foods	Dry Matter	Protein	Lipid
Snail Meat	21.82	13.74	0.57
Beef	42.7	24.4	15.10
Mutton	36.3	18	17.5
Fish Meat	22.8	19.0	2.5
Egg	26.0	12.8	11.5
Milk	13.0	3.5	3.9

Due to their low cholesterol and fat content and high mineral and protein content, snails are considered one of the healthiest diets for humans (Özoğul et al., 2005). Snail meat has a low calorie count (67 kcal/100 g), more nutrients than even the leanest meat or fish, and is a great diet meal because of all of these things. In terms of microelements, snail meat has ten times more calcium than conventionally consumed meat. Additionally, snail foot muscles are known to be exceptionally rich in iron, copper, zinc, and selenium, an essential antioxidant (Duman, 2015).

According to research by Düzgüneş et al. (1992), sea snail meat generally consists of 72.04% water, 16.29% protein, 2.25% fat, and 1.82% ash. Stoeva et al. (1995) found that the same species had a carbohydrate content of 8.9% (glycogen).

Before and after cooking, Merdzhanova et al. (2018) conducted research on the lipid composition, fatty acid content, fat-soluble vitamin content, and cholesterol content of sea snails. The cooking process resulted in a considerable alteration in the composition of fatty acids, but the temperature had no effect on the fatty acid groups that were present in phospholipids. Palmitic acid (C16: 0) and eicosapentaenoic acid (C20: 5n-3) were the fatty acids that were found in the highest concentrations across all lipid classes in both raw and cooked samples. According to the findings of this research made by Merdzhanova et al. (2018), cooking has an effect on the amount of fat-soluble cholesterol in the flesh.

In a study conducted by Arslan (2009), the alteration in nutritional properties resulting from the use of various processing procedures to sea snails was examined. To boil sea snails, begin by pre-boiling them at a temperature of 105°C for a duration of 15 minutes, followed by a subsequent boiling at 110°C for 40 minutes. Following the boiling phase, the product underwent various processing techniques. These included pasteurization, which involved subjecting the product to a temperature of 90 °C for 15 minutes, canning, which involved subjecting the product to a temperature of 121 °C for 20 minutes, smoking, and marinating at a temperature range of 70-80 °C for 2 hours. Additionally, fresh sea snails were examined separately, and the analysis results were provided. The moisture content of fresh sea snail, boiled at 105 °C for 15 minutes, boiled at 110 °C for 40 minutes, was found to be 71.30% ±0.05,

69.74% ±0.21, 70.63 ±0.17%, respectively. The protein result of the fresh sample, boiled for 15 minutes at 105 °C and boiled for 40 minutes at 110 °C was determined as 19.55±0.45%, 20.18±0.00%, 21.98±0.00%, respectively. Based on the results of the lipid oil analysis, the fresh sample had a lipid content of 0.45 ±0.10%, the sea snail cooked for 15 minutes at 105 °C had a lipid content of 0.24±0.03%, and the sea snail boiled for 40 minutes at 110 °C had a lipid content of 0.31±0.04%. The lipid content of sea snails boiled for 15 minutes at 105 °C and 40 minutes at 110 °C was found to be significantly lower than that of fresh snails, although there was no statistically significant difference between the two.

Table 2 displays the results of the nutritional evaluation of sea snail products processed using various methods.

Table 2. The nutritional content of sea snail products made using various processing methods (%) (Arslan, 2009).

	Pasteurized Snail	**Canned Snail**	**Smoked Snail**	**Marinated Snail**
Moisture	72.46±0.28^{a}	66.75±0.49^{b}	51.54±0.77^{c}	69.37±0.37^{d}
Protein	19.64±0.20^{a}	23.54±0.10^{b}	31.35±0.37^{c}	19.55±0.06^{a}
Lipid	0.21±0.06^{a}	0.51±0.02^{b}	0.93±0.08^{c}	0.26±0.02^{a}
Ash	2.56±0.04^{a}	2.50±0.03^{a}	7.44±0.04^{b}	3.50±0.05^{c}

In his 2015 research, Kıran investigated the impact and variations of sea snails collected from 12 distinct locations in the Eastern Black Sea (Giresun, Trabzon, Rize, and Artvin provinces) using scuba and free diving techniques. The study focused on analyzing the dry matter, ash values, crude protein, and crude lipid levels of sea snail meats throughout various seasons. Despite the fact that the characteristics that have been studied differ from one location to another, the overall average values of the seasons were as follows: dry matter (%), ash values (%), crude protein (%), and crude lipid (%) quantity have been found to be 24.69%, 2.29%, 16.43%, and 0.58%, respectively.

Popova et al. (2017) examined the variations in the characteristics and lipid composition of snail meat (*Rapana venosa*) during different seasons. The snails used in the research were collected by divers from the Bulgaria (Bay of Varna) area, located one mile away from the coastline, at a depth ranging from 10 to 15 meters, starting from late spring (June-October). The snails that were gathered were measured using a digital scale. Furthermore, the tissue, operculum, and intestines were extracted from the shell, and the weight of the flesh was documented. During the months of June, July, and October, the researchers discovered that the snail's live weight ranged from 56.44 to 110.02 grams, and that the meat content was compatible, ranging from 11.98 to 23.27 grams. Analysis revealed that the meat output reached its minimum in June and thereafter exhibited a rise in July. However, in the subsequent samples obtained, the meat yield gradually declined until October. Research on the quality of snail (*Rapana venosa*) meat found that chemical analyses revealed a moisture content ranging from 70.89% to 76.24%, with the greatest value recorded in July and the lowest value recorded in October. July had the lowest protein value and October had the highest, with a range of 18.62% to 24.09%. Lipid analysis shows that it has modest levels, ranging from 0.58% to 0.85%.

Kocabaş and Fenercioğlu (1992) investigated the alterations detected in land snails (*Helix pomatia*) obtained from different areas of Adana, which were then stored and operated on at a commercial company. The land snails that were gathered and brought in throughout the months of April, May, and June in 1989 were maintained in a living state for durations of 0, 1, 2, and 3 days. The results revealed that the total dry matter content exhibited variation based on the months during which the samples were collected. The relative dry matter content was determined to be 20.84%, 21.54%, and 23.21%. The snail meat had fat contents of 0.61%, 0.34%, and 0.76% correspondingly. Upon protein analysis, snail meat was shown to be a significant source of protein, with an average content of 13.74%. The protein contents varied by month, with values of 13.94%, 14.1%, and 13.18%. The average ash content of snail meat varied by month, with values of 0.26%, 0.28%, 0.24%, and 0.25% recorded for each corresponding month.

Olgunoğlu and Olgunoğlu (2009) examined the chemical composition values of snails (*Helix lucorum* Linnaeus, 1758), collected from different geographies of Turkey and brought to the snail processing factory, as they were prepared for consumption by weighing them. The analyzes of the research were carried out after thawing and homogenizing the snail samples in butter sauce prepared by adding parsley and garlic, frozen at -40 °C, in the refrigerator. As a result of the study, the moisture, ash, crude protein, lipid, saturated fat and unsaturated fat of snail meat, ready for consumption, were found to be 54.77%, 2.57%, 10.22%, 27.91%, 16.77%, and 8.86%, respectively.

The nutritional value of sea snail (*Rapana venosa*) meats was evaluated at each step of processing by Korkmaz and Pinal (2022) using a fisheries processing facility. Each stage included scalding, washing, skin removal, calibrating, freezing at -40 ^{o}C after 1 hour, and storage at -18 ^{o}C after 1 week. Based on the study, the raw moisture contents throughout the several phases of processing were determined to be as follows: Besides, the

raw ash content contents were determined as 73.32±0.90% (scalding), 70.53±0.17% (washing), 72.67±0.27% (skin removal), 75.51±0.57 (calibration), 76.79±0.14% (freezing), and 76.01±0.10% (frozen storage). In addition, the following raw ash content contents were found: 2.49±0.03% for scalding, 2.31%±0.27% for washing, 2.74±0.50% for skin removal, 1.25±0.14% for calibration, 1.08%±0.16% for freezing, and 1.09±0.14% for frozen storage. Moreover, crude protein and lipid contents were calculated as 19.44±0.07% and 0.83±0.52% (scalding), 21.46±0.70% and 1.65%±0.65% (washing), 20.07±0.08% and 0.53±0.0% (skin removal), 18.92±0.72% and 0.53±0.00% (calibration), 19.28±0.57% and 1.08±0.16 (freezing) and, 18.17% ±0.41 and 1.09±0.14% (frozen storage), respectively.

4. Conclusion

Sea snails meats are an important source of income for the people of the Black Sea region and are an important export product as they are in demand abroad. It provides a source of income for tens of thousands of people, from hunting by boats and divers to its transportation from processing factories. It will be possible for Turkey to reach a higher potential in snail exports and increase its market share in snail trade by obtaining suitable quality products. In snail hunting, collection conditions need to be improved, transportation under appropriate conditions must be monitored, and processing processes must be improved. It is important to reveal the processing process and changes in nutritional and quality parameters of sea snail, which is an important export product for the Black Sea.

Conflict of Interest

The authors have no conflict of interest to declare.

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REVIEW ARTICLE

Electrophoretic Methods for Identifying the Species of Seafood and Its Derivatives

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ABSTRACT

The identification of the species of seafood and their products, whether they are fresh or cooked, is one of the key concerns of food regulations in many countries that have a significant intake of seafood. In point of fact, a commercial fraud happens when a species that is less value is intentionally substituted for a species that is more valuable, and a sanitary fraud takes place when a product that is potentially harmful is introduced into the market. A primary responsibility of veterinary inspection of seafood products is the detection of harmful species with the aim of removing them from the retail trade (Council Directive 91/493/ECC). Two efficient methods for seafood species identification are molecular biology methods and protein electrophoresis. Classic electrophoretic methods have been used for a long time to authenticate seafood; they are simple, accurate, and inexpensive compared to molecular biological methods, which are the wave of the future in food safety labs. The purpose of this article is to offer an overview of the electrophoretic methods commonly used to identify different species of seafood, including sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), native or urea-isoelectric focusing electrophoresis (IEF), two-dimensional electrophoresis (2-DE), and capillary electrophoresis (CE).

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1. Introduction

Assuring proper species identification, mode of production (cultivated or wild) and geographical origin, via "seafood authentication" verifies that seafood items have been labeled correctly. The most important part of seafood authentication is species authentication, which helps keep seafood fresh, and prevents fraud by guaranteeing market transparency. By using species identification, we can be sure that the product's commercial and scientific names are accurate representations of the species (Boziaris, 2014).

Species identification of fishery and aquaculture products is essential for ensuring fair business practices and providing accurate consumer information. According to the European Union has established European Commission Regulation (EC) 104/2000 (EC, 2000) and 2065/2001 (EC, 2001), fish products are only allowed to be sold commercially provided the label clearly states the commercial name, method of production, and catch area. When dealing with cultivated species, it is necessary to provide the appellation of the country name of the nation in which the product will be subjected to the ultimate stage of its growth. When these standards are followed, consumers are given more options to choose from, and they are provided with complete and accurate information about the product. These regulations may be successfully implemented provided comprehensive species-specific data for all fish species are accessible, in order to avoid the replacement of economically significant fish.

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The challenges associated with identifying fish species in fish products are attributed to many reasons, notably (Civera, 2003):

- The process of market globalization has led to a significant increase in the number of various species that need to be studied.
- A significant quantity of processed fish products that do not possess the morphological features necessary for the conventional identification process.
- Inadequately trained individuals used in the identification of species.
- Labelling fish species is essential to prevent adulteration, as it helps to avoid fraudulent substitution of fish species with varying values and prices.

The range of fish items available on the market is quite varied. Furthermore, they provide an assortment of processed fish goods, including fish balls, fish steaks, canned fish, and other similar items. Also, given that the majority of fish are imported in the form of compressed flesh blocks or fillets, it is not feasible to identify them based on surface features Due to the advancement of processing technology, the conventional practice of using fish appearance to identify species has become less successful, leading to a potential rise in the occurrence of substitutes. The primary approach for identifying fish species is explicitly differentiating them based on their physical traits or morphology, including skeletal structure, muscles, branching taxonomy, as well as fin characteristics, scales, and life history. Nevertheless, the visual attributes of intact fish sometimes prove inadequate for precise differentiation of fish species, and the identification of processed fish products poses an even greater challenge (Chien et al., 2022).

Protein analysis methods center on a number of physical distinctions, including size, net charge, and amino acid content, among others. In the late 1970s, the first protein markers were created by determining the water solubility of proteins and isozymes using electrophoresis in polyacrylamide or starch gels. These markers paved the way for new understanding of the genetic diversity and structural variation across populations, species, and variations hailing from all over the world. As a result of the fact that proteins are the final product of gene expression, which can differ from tissue to tissue, developmental stages, environments, and seasons, this method has a significant drawback: it couldn't detect enough variation to distinguish between related varieties or species. (Hubalkova et al., 2007; Lago et al., 2014).

Protein electrophoresis and molecular biological methods are the two primary approaches to species identification in cases where the fish has lost its biological characteristics (Etienne et al., 2001). Classic electrophoretic methods have proven to be reliable, easy to apply by food control laboratories, and, at the moment, still less sophisticated and less expensive than molecular biological methods (Piñeiro et al., 1999a, 1999b), despite these latter methods representing the future in food control laboratories. Several electrophoretic methods are available, and the choice of methodology relies on the required level of resolution and the type of the material being analyzed (e.g., fresh, frozen, heat treated, etc.). To prevent the adulteration of fish species and protect consumer rights, **f**ish species have been successfully identified using classic electrophoretic, such as sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), native or urea-isoelectric focusing electrophoresis (IEF), two-dimensional electrophoresis (2-DE), and capillary electrophoresis (CE) (Rehbein et al., 1999; Civera, 2003; Hubalkova et al., 2007). For the purpose of identifying the species of raw and heat-processed fish, Piñeiro et al. (1999a) and Etienne et al. (1999) optimized the standard operating procedures of SDS-PAGE and urea-IEF (Mackie et al., 2000; Etienne et al., 2001a, 2001b). The protein electrophoresis technology remains more cost-effective and less complex to run compared to DNA-based approaches (Piñeiro et al., 1999a, 1999b). Table 1 shows various methodologies based on protein analysis for the identification of seafood (Civera, 2003).

Table 1. Different approaches to seafood identification based on protein analysis (Civera, 2003).

Fishery product	Methods	Compounds to be analyzed	Drawbacks	Reference
Raw fish and raw products	Electrophoretic $ IEF	All sarcoplasmic proteins	Heat denaturation; polymorphism	Durand et al. (1985), Rehbein (1990), Rebhein et al. (1995), Colombo et al. (2000), Tepedino et al. (2001)
Raw and processed fish	$ IEF	Acid, calcium-binding proteins (parvalbumins)	High concentration in white muscle	Esteve-Romero et al. (1996)
	Specific enzymic detection, IEF	Enzymes in sarcoplasmic proteins (LDH and G-3-PD)	Heat denaturation	Piñeiro et al. (2001)
	Capillary zone electrophoresis	Sarcoplasmic protein	Heat denaturation	Larrain et al. (2002)
Heat-processed	SDS-PAGE	Sarcoplasmic and myofibrillar proteins	Faint variability in fish protein pattern (no identification in closely related species)	Civera and Parisi (1991), Etienne et al. (2001)
	Urea-IEF	Sarcoplasmic and myofibrillar proteins	Good identification only in species rich on parvalbumins	Etienne et al. (2001)

2. Native or Urea-Isoelectric Focusing Electrophoresis (IEF)

The method of isoelectric focusing of sarcoplasmic proteins, which make up 20 to 35% of fish muscle proteins, is well recognized for species identification of seafood, and its reliability has been evaluated in several research (Rehbein, 1995; Mackie, 1996). Arginase (ARGK), arginin kinase (CK), malate dehydrogenase (MDH), adenylate kinase (AK), aldolase, glycerol 3-phosphate dehydrogenase (G 3-PD), lactate dehydrogenase (LDH), and other proteins like parvalbumin, myoalbumin, and globulin are all part of the sarcoplasmic proteins. Because various fish species exhibit different band patterns when separated using the isoelectric focusing method, the proteins found in the sarcoplasmic fraction are ideal for this purpose (Recio et al., 2001). The process of sarcoplasmic protein separation in IEF is achieved by using their isoelectric point, employing distinct pH ranges such as 'wide range' (3-10) and 'narrow range' (3-6) (Civera, 2003). The IEF approach relies on the separation of molecules on a gel, which is accomplished by adding an ampholyte to create a pH gradient (Hubalkova et al., 2007). Specifically, the anode may be used for species identification by using the acidic proteins that are located nearby, with pI values ranging from 3.5 to 5.5. These proteins are small in size and have the feature of binding to calcium (Verrez-Bagnis et al., 2017).

Many fish species for species identification of nonheat treated fish musculature and products that have been identified using IEF technology includ; snapper, catfish, *Sparidae* species, tilapia, barramundi, different Atlantic and Aegean species, spearfish and swordfish, red snapper, puffer fish, tunas, mackerels, bonitos, flat fish and perch species in combination with two-dimensional electrophoresis or *gadoid* species through the use of auxiliary enzyme detection in the detection of food fraud (Verrez-Bagnis et al., 2017). Most sarcoplasmic proteins may be dissolved in aqueous solutions and undergo denaturation when exposed to heat treatment. Rehbein et al. (1999) demonstrated that IEF approach is more effective than conventional electrophoresis in distinguishing sarcoplasmic proteins due to its greater selective capacity. The IEF method was used to construct a library of commercial fish species that were classified as either *Gadiformes* or *Pleuronectiformes* based on the sarcoplasmic proteins they contained (Tepedino et al., 2001). It was possible to differentiate gadoid fish species due to the presence of certain enzymes in the interferon-enzymatic fraction (IEF) of sarcoplasmic proteins in the pH range of 3.5-9.5. They are ARGK, LDH, G 3-PD, AK, CK, and MDH, among others. The mobility of LDH and G 3-PD on electrophoresis was used to discriminate most of the gadoid fish species investigated by Piñeiro et al. (2001), including closely related members of the *Merlucciidae* family. Ataman et al. (2006) also employed the IEF method of sarcoplasmic proteins to distinguish between nongadoid and gadoid species; a related methodology has been developed.

Protein configuration is additionally impacted by freezing because the amount of water that is available in frozen products decreases. As a result, proteins tend to aggregate and precipitate, which makes it difficult to extract proteins and obtain reproducible results from the methodology (Shenouda, 1980).

Examinations of fish from the genera *Thunnus* or *Tetrapturus* revealed similar patterns among closely related species. Occasionally, protein polymorphism leads to variances in patterns among specimens of the same fish species, which makes it difficult to assign samples. The charge polymorphism of proteins, observable by IEF, may be affected by either genetic variables (such as the expression of different alleles of a single gene) or by post-translational metabolic processes (e.g., phosphorylation). Various protein patterns have been recorded in several species of fish and mollusks. Polymorphism poses difficulties and impedes food management efforts, although it also forms the basis for the electrophoretic separation of distinct populations of fish species. In order to accomplish this objective, a range of isoenzymes are extracted (often by electrophoresis on starch gels) and rendered detectable using staining methods specific to each enzyme. To provide reliable and accurate outcomes from IEF of fishery products, it is essential to consider several factors that might influence protein patterns (Rehbein, 1990).

Two varieties of fish may be recognized based on the color of their fillet: white-fleshed fish (such as cod and plaice) and red-fleshed fish (such as sardine, mackerel, and tuna). Fishes belonging to the latter category possess a greater amount of myoglobin and a larger proportion of red muscle compared to fishes with white meat. White and dark muscle exhibit distinct variations in their physiological function and metabolic processes. White muscle is distinguished by elevated levels of glycolytic enzymes, reduced quantities of chromoproteins, and a high concentration of parvalbumins. Hence, it is unsurprising that the protein patterns of both muscle types exhibit variations in terms of the quantity, location, and intensity of protein bands when subjected to analysis using IEF. White muscle may often be differentiated from red muscle by its prominent anodal bands, which are indicative of parvalbumins and perhaps additional unidentified acidic proteins. Both forms of muscle exhibited distinct protein patterns that were unique to each fish species. With the exception of a few cases, red muscle has not been used for the purpose of species identification. Based on the above statements, it is evident that in order to get consistent findings using electrophoresis, it is necessary to separate white muscle from blood, red muscle, or any other tissues (Rehbein, 1990).

Identification of species is done by analyzing the parvalbumin pattern or the whole protein pattern. In the white

muscles of several fish species, including *Pleuronectiformes* and *Gadiformes*, you may find parvalbumins, which are acidic, calcium-binding, heat-stable sarcoplasmic proteins with a low molecular mass (12 kDa) (Esteve-Romero et al., 1996). Identifying a species of fish is as simple as comparing its pattern to a standard. The USFDA has produced a Regulatory Fish Encyclopedia that includes IEF patterns of sarcoplasmic proteins from 76 species that are widely sold in North America (Available online at *https://wayback.archive-it.org/7993/20170406002931/https://www.fda.gov/Food/FoodScienceResearch/RFE/ucm219129.htm*). Unfortunately, there are currently no protein patterns for toxic seafood. Not only has IEF been used effectively on raw fish, but it has also been used to cooked fish, smoked fish products, and sturgeon eggs. The proteins are dissolved using urea or SDS, and the electrophoretic pattern is stained with silver (Etienne et al., 1999; Piñeiro et al., 1999a; Rehbein et al., 1999).

Based on their research, Renon et al. (2005) concluded that IEF is an easy and dependable way to spot blue marlin steaks that have been interchanged for Mediterranean spearfish (*Tetrapturus belone*) and cold-smoked swordfish (*Xiphias gladius*) fillets that have been fraudulently switched out for lower-value blue marlin (*Makaira mazara*).

Regarding the application of IEF to shrimp authentication, a study with sarcoplasmic proteins succeeded to differentiate generic shrimp from fish and lobster (Rehbein, 1995; Ortea et al., 2010). The specific proteins, which belonged to the class of calcium-binding polypeptides, were in the 4-5 pI range, and resulted to be heat stable. For this reason, such proteins provided species-specific patterns for cooked products as well as in raw samples. In the same work, a similar method based on IEF in urea gels was applied to the analysis of products in which the sarcoplasmic proteins had been previously removed by washing steps during processing, this strategy allowed the differentiation of shrimp from fish and cephalopod meat. Although specific bands were detected in heated samples, the shrimp species could not be differentiated due to protein loss and band clustering (Ortea et al., 2012).

Proteins are denatured and precipitated during heat treatments. Due to the difficulty in identifying species using electropherograms of the sarcoplasmic proteins, this method is only useful for analyzing foods that are either slightly cooked, frozen, or very fresh. To employ electrophoretic methods in cooked foods, one must first extract the precipitated proteins using urea, which enables denaturing-condition protein extraction (Boziaris, 2014). According to Ortea et al. (2012), several studies have shown that IEF is not appropriate for heated or marinated food because it causes the breakdown of some muscle proteins under these settings.

Urea-isoelectric focusing (An et al., 1988) can be used to analyze heat-denatured muscle protein that has been extracted using denaturing solvents containing urea. Rehbein et al. (1999) and Etienne et al. (1999) optimized the urea-IEF methods for identifying cooked fish species. The IEF approach can only be used for heated products of fish species that exhibit a distinct pattern with the heat-stable parvalbumins (Rehbein, 1992). In general, the process of heating may cause the denaturation of myofibrillar and sarcoplasmic proteins in fish muscle. To solubilize these proteins, a chaotropic agent like urea or a detergent like sodium dodecyl sulfate (SDS) can be used. The proteins generated using this method may be further examined using urea-IEF (An et al., 1988).

Etienne et al. (2001) conducted a comparative analysis of *Gadus morhua*, *Theragra chalcogramma*, *Pollachius virens*, *Macruronus novaezelandiae*, and other fish species in terms of their electrophoretic distinguishability using the urea-IEF, SDS-PAGE, and native IEF procedures. Each of the three methods effectively distinguished between different types of processed fish. However, SDS-PAGE and only urea-IEF were shown to be useful in identifying fish species in products treated with high pressure. However, it was also shown that urea-IEF is less precise than SDS-PAGE in distinguishing between salmonoid fish and tuna fish, since the proteins of the latter exhibit neutral or alkaline properties. SDS-PAGE failed to differentiate between *Gadus morhua* and *Theragra chalcogramma*, despite its ability to differentiate other gadoid fish species. According to authors, if proteins remain in their original structure (not denatured), native IEF may be used.

Denaturing urea-IEF has shown better efficacy in identifying species in high pressure-treated products (Etienne et al., 2001). It may also serve as an alternate method for determining the species of fried fish, depending on the specific product (Rehbein et al., 1999; Etienne et al., 2001; Verrez-Bagnis et al., 2017).

According to Etienne et al. (1999), proteins undergo denaturation and degradation in various processing techniques as cooking, frying, and smoking. As a consequence, the distinctive bands seen in IEF are lost. Regarding the products provided, we provide gravad and smoked salmonid items, as well as smoked eels., the findings of a collective investigation indicate that urea IEF is more efficient than SDS-PAGE in distinguishing between different species. (Mackie et al., 2000). The relevant research group generally suggested a mix of native IEF, urea IEF, and SDS-PAGE, with the specific ratio varying by product and species. Protein band patterns were sufficiently similar that IEF alone could not distinguish several closely related species (Verrez-Bagnis et al., 2017).

Lago et al. (2014) stated that IEF and urea-IEF provide some benefits over conventional electrophoretic techniques:

- Electrophoresis results in the proteins being confined to small zones, leading to enhanced resolution and sensitivity.
- After electrophoresis, the system reaches equilibrium, reducing the impact of any changes in the experimental

settings on the resulting protein pattern. The bands of the sample can then be compared to the bands of the reference patterns using images.

- Utilizing polyacrylamide gels produced by companies in conjunction with semi-automated machinery enhances the consistency of the outcomes and reduces the duration of analysis.

Additionally, they indicated that these IEF approaches exhibit significant disadvantages as well:

- The resulting protein profiles include several bands, making their interpretation sometimes challenging.
- This arduous approach requires skilled operators and appropriate equipment.
- The sample procedure is intricate and the technology is costly, complicating its use in laboratories that conduct food analyses (Lago et al., 2014).

3. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE is the most often used method for achieving precise separation of protein mixtures with high resolution. The process comprises the first denaturation of constituent proteins using an anionic detergent that also binds to them, resulting in all proteins acquiring a negative charge that is directly proportionate to their molecular mass. Following this, electrophoresis is conducted using a permeable acrylamide gel matrix, which effectively separates proteins based on their molecular mass with high precision (Walker, 1996). The SDS detergent is appropriate for extracting denatured proteins in studied materials, making it a good approach for determining species that have undergone heat treatment (Članjak–Kudra et al., 2021).

Myofibrillar proteins, sarcoplasmic proteins, and total muscle homogenates are the three types of muscle extracts that SDS-PAGE has been proposed by many authors as an alternative to IEF methods. Certain items, such as imitation crab sticks, crab claws, and lobster tails, were specifically required to undergo SDS-extraction. These products are crafted from minced fish flesh (surimi), which has been extensively washed to remove the majority of water-soluble proteins. Peptide mapping of the myosin heavy chain employing restricted proteolysis for fish identification was suggested as a solution to tackle the most challenging cases (Esteve-Romero et al., 1996).

Gel electrophoresis with sodium dodecylsulfate is a useful tool for analyzing heat-denatured muscle protein that has been extracted with denaturing solvents including urea or SDS. Solution methods like 2% sodium dodecyl sulphate (SDS) allow for the dissolution of heat-denatured proteins, which may then be separated using SDS-PAGE. Methods for identifying species of cooked fish using SDS-PAGE were improved by Rehbein et al. (1999) and Etienne et al. (1999). When employing the urea-IEF and SDS-PAGE methods for fish species differentiation, proteins with a low molecular weight, such as myosins (14-23 kDa), troponins (19-30 kDa), and parvalbumins (12 kDa) are the most suitable options (Etienne et al., 1999; Piñeiro et al., 1999a; Rehbein et al., 1999). Additionally, SDS-PAGE has shown improved species identification in high pressure-treated products (Etienne et al., 2001), and there may be different methods for cooking fish species depending on the product type (Rehbein et al., 1999; Etienne et al., 2001; Verrez-Bagnis et al., 2017). But it is inefficient for differentiating between canned fish species (Mackie et al., 1999).

While Civera and Parisi (1991) did find some subtle variation in SDS-PAGE patterns across generic shrimp and crabs, they were unable to distinguish between individual species within either group. Pink shrimp (*Farfantepenaeus duorarum*), white shrimp (*Litopenaeus setiferus*), and rock shrimp (*Sicyonia brevirostris*) were successfully distinguished using SDS-PAGE, as shown by An et al. (1988). Specific electrophoretic patterns for each species were observed in the water-extractable protein fraction of the raw specimens. Protein precipitation made species identification impossible when water-soluble samples were heated; under these conditions, only an extraction with SDS allowed for the separation of the two genera (Ortea et al., 2012).

Using SDS-PAGE protein patterns, Rouxel et al. (2001) determined that four different types of seaweeds could be identified. Species such as *C. crispus* and *G. verrucosa* stand out as having quite different characteristics when compared. That notwithstanding, SDS-PAGE seems to be a practical way to identify red seaweeds that are approved for usage as sea veggies or food components. Additives like carrageenans and agar are also made from bacteria like *C. crispus* and *G. verrucosa*. It is possible to regularly utilize electrophoresis on raw materials for food phycocolloid manufacturing. However, Fleurence et al. (1995) demonstrated that the technology was specifically tested on raw material SDS-PAGE and effectively used under certain circumstances to differentiate between two *Ulva sp.* that are often eaten as sea vegetables.

The procedures for protein extraction from the SDS solution and SDS-PAGE electrophoretic analysis were detailed by Piñeiro et al. (1999a). Salmonid fish that had been smoked or marinated could not be positively identified using the SDS-PAGE method. There was hard to distinguish the Atlantic salmon (*Salmo salar*) from the smoked sea trout (*Salmo trutta*) since they seemed so identical as well. The rainbow trout (*Oncorhynchus mykiss*) did show some variation, but the differences found were not strong enough to be identified with certainty. Differentiating smoked arctic char (*Salvelinus alpinus*) from other processed fish was a breeze. But we were able to identify every single raw reference species. The SDS-PAGE method was ill-suited for detecting treated substances

because the changes in protein band separation were very minute. There is little effect of smoking and marinating on the protein separation achieved by SDS-PAGE and IEF using urea methods. Because of this, it is somewhat more difficult to distinguish between closely related species.

The species of salmon and trout may be identified by native electrophoretic, SDS-PAGE, and IEF, as stated by Mackie et al. (2000). Although there are more protein bands produced by the SDS-PAGE technique compared to the IEF method for processed items, there are still not enough unique bands to reliably identify them. Therefore, when trying to differentiate between salmon and trout, the IEF method is widely regarded as the most effective. Arctic char and rainbow trout, on the other hand, were determined to be more suited to the SDS-PAGE method. Nevertheless, the previous studies have shown that eel species may be identified using both SDS-PAGE and IEF with urea procedures. In contrast, the urea-based IEF technique works better for distinguishing between the two Atlantic eel species, *Anguilla rostrata* and *Anguilla anguilla*. The indigenous IEF method is another option for differentiating the Atlantic short-finned eel (*Anguilla australis*) from its southern version. However, given the many situations surrounding smoking in the workplace, this approach just provides supplementary data (Mackie et al., 2000).

The SDS-PAGE method was used by Martinez et al. (2001) to determine the species of Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), minke whale (*Balanoptera acutorostrata*), Arctic char (*Salvelinus alpinus*), and harp seal (*Phoca groenlandica*). They used a variety of gels in order to conduct an analysis on samples that had not been treated in addition to those that had been processed (cold and hot smoked, cooked, and marinated). The researchers observed that the Anderson gel exhibited remarkable proficiency in distinguishing Atlantic salmon and rainbow trout by displaying six clearly identifiable diagnostic bands. The ExcelGel exhibited three clearly distinguishable bands, but the Tris-Tricine gel showed two bands and the NuPAGE Bis-Tris gel exhibited just one band. All experimental gels, except for the ExcelGel, exhibited similar profiles when subjected to testing using the same species in both their raw and cooked forms. Additionally, it was observed that the distinctions between the marinated and smoked samples were notably different from the raw samples, resulting in increased difficulty in their identification. The achieved differentiations were inadequate to discern between the smoked and marinated rainbow trout and salmon. Furthermore, the bands that migrated the quickest exhibited blurring and warped paths on most of the gels. Estimation is an important procedure, but it should be noted that the findings may vary somewhat for the same bands based on factors such as migration lengths, temperature, buffer pH, etc. For accurate assessment of a product's authenticity, it is crucial to examine samples on the same gel, under same conditions.

Huang et al. (2010) conducted a study to determine the specific species of moray eel responsible for cases of food poisoning. The moray eel meat, along with eight other raw commercial moray eel meats (*Gymnothorax favagineus, G. fimbriatus, G. flavimarginatus, G. meleagris, G. pseudothyrsoideus, G. undulates, G. albimarginatus*, and *G. javanicus*), were subjected to a causative process by heating them at 100 ℃ for 30 minutes. The samples were then analyzed using the electrophoresis method known as SDS-PAGE. The SDS-PAGE analysis of eight processed commercial moray eel meats revealed distinct protein bands with a molecular weight of less than 30 kD, which were peculiar to each species. The specific causative species of moray eel involved in the food poisoning case was determined to be *G. javanicus*. This conclusion was based on a comparison of the protein extracted using 2% SDS and 8 M urea, as evidenced by the SDS-PAGE patterns.

Lin et al. (2012) identified the poisonous sample as *L. bohar* based on the low molecular weight region (<30.0 kD) of both the myofibrillar and sarcoplasmic patterns. Ordinary SDS-PAGE analysis proved effective in achieving species identification, as indicated by the results.

4. Two Dimensional Electrophoresis (2DE)

2-DE enables the simultaneous separation of a large number of proteins in a single gel. In the first dimension, proteins are separated using the native technique, typically IEF, based on their PI. In the second dimension, proteins are separated using SDS-PAGE based on their MW. This method offers several advantages. Firstly, it has the capability to separate a large number of proteins simultaneously. Additionally, it can effectively separate proteins in the sample from unwanted substances through protein precipitation. Moreover, it enables the examination of similarities and differences between proteins extracted from muscles of various animal species. (Hofmann, 1997; Alikord et al., 2018).

The study conducted by Piñeiro et al. (1998) examined the profiles of water-soluble proteins in eight species of gadoid fish, including five hakes (*Merluccius spp*.), cod (*Gadus morhua*), pollack (*Pollachius pollachius*), and blue whiting (*Micromesistius poutassou*), were examined using 2D electrophoresis. Using the findings from the 2D mixed-extract analysis, they identified that among the species of *Microcystis*, one parvalbumin (10.4 kDa, pI 3.9) was shared with *M. australis* and *M. hubbsi*, while two parvalbumins (10.3 kDa, pI 4.1; 10.4 kDa, pI 3.9) were shared with *M. capensis*. They concluded that 2D electrophoresis could provide a wealth of information about the molecular weights and isoelectric points of the primary water-soluble proteins, which would be useful for distinguishing between closely related species of *Merluccius*.

Piñeiro et al. (1999b) used two-dimensional (2D) electrophoresis, gradient SDS-PAGE in the 12-14% range, nondenaturing isoelectric focusing (IEF) in the 3.5-9.5 pH range and to examine the water-soluble proteins of nine economically valuable flatfish species from the families *Pleuronectidae*, *Scophtalmidae*, and *Soleidae*. Between 3.5 and 6.9 pH, the majority of the major proteins were found. Out of all of these, the acidic fraction contained the proteins with the highest specificity (MW<16 kDa; pI<5.2). In all nine species, they found 2D protein patterns that were specific to that species, and we were able to personalize over 25 proteins. Nondenaturing IEF and gradient SDS-PAGE, performed in a urea-free environment, provided an effective method for differentiating between flatfish species under study by allowing for the precise determination of molecular weights and isoelectric points of the main water-soluble proteins.

The *Merluccidae* family is comprised of seafood species, some of which have a significant economic worth while others have a lower value. The commercialization of the *Merluccidae* family in Europe under the generic name "hake" is extremely remarkable, as was studied by Piñeiro et al. (2001). In order to achieve the goal of achieving the differential characterization of five different species of hake, the potential of proteomics was utilized in this study. These species include *Merluccius australis* (Southern hake), *Merluccius merluccius* (European hake), *Merluccius gayi* (Chilean hake), *Merluccius hubbsi* (Argentinian hake), and *Merluccius capensis* (Cape hake), some of which are intimately associated with one another. Four of the five species of hake that were investigated using IEF and/or 2-DE high-resolution gels were found to contain polypeptides that were specific to their species (Rodríguez & Ortea, 2017).

Berrini et al. (2006) used two-dimensional electrophoresis (2-DE) to differentiate between different commercial values of four freshwater fish species that are sold under the generic name "perch" and are easily "interchangeable" because they are already filleted: *Perca fluviatilis*, *Lates niloticus*, *Stizostedion lucioperca*, and *Morone chrysops* x *saxatilis*. Multiple species-specific spots on the proteins were shown by the 2-DE maps. Oddly enough, all four species shared multiple noticeable 2-DE spots, but none of them shared IEF bands. Analysis by 2-DE, a more expensive and time-consuming method with better resolution power, was determined to be an effective means of extracting more information from the proteomes of understudied species.

Two-dimensional electrophoresis (2DE) is a technique that requires specialized staff and is characterized by the complexity of the obtained profiles. Additionally, it is a time-consuming and labor-intensive process. Therefore, it has not been employed for the identification of numerous species. Classification of species in raw and cooked aquatic foods has been accomplished through the utilization of the 2DE method, in conjunction with its constituent techniques. (Valenzuela et al., 1999; Chen et al., 2004; Berrini et al., 2006).

5. Capillary Electrophoresis (CE)

CE, is a method that combines electrophoresis and chromatography and it has been utilized for the purpose of species differentiation. The variation in electrophoretic mobilities of the compounds under investigation within a high-potential-exposed narrow-silica capillary column (50-150 μm in diameter) determines the outcome. While the mass-to-charge ratio does not change, the molecules are categorized based on their different masses (Članjak–Kudra et al., 2021). According to Lago et al. (2014), this automated method can be utilized for commercial purposes due to its relatively fast analysis time of only about 10 minutes. Recio et al. (2001) utilized various variations of CE in their investigation of animal protein analysis. One approach used sarcoplasmic protein analysis in fish musculature to rule out gadoid species. The methods described above were used to identify all species of salmonid fish and smoked eels (Mackie et al., 2000).

When compared to various other methods of analysis, this method has the following advantages:

- It can detect and quantify multiple molecules at once thanks to a system that automatically replaces the column's filling buffer with another one, eliminating the need to manually handle each component of a sample during analysis.
- The entire analysis process takes less than 10 minutes, with just 3 minutes needed to clean and rebalance the column in between each set of analyses.
- Minimal volumes of samples are necessary. Although this method was insensitive at first, that is no longer an issue thanks to enhanced detection systems.

But, its primary drawback is that:

- Each reagent requires its own unique detection system, which, because of the extremely small volumes used, must be extremely sensitive (Westermeier, 2016). Using CE for protein analysis is a fully automated process. Compared to other electrophoretic methods that are currently available, this one has a significant advantage because it doesn't require specialized technicians and is easier to perform (Lago et al., 2014).

6. Conclusion

There is no doubt that there will be an increase in the number of instances of food fraud all over the world. This is due to a number of factors, including the rise in population, the shifting trends in nutrition, the desire to make a profit, and the growing demand for fish as a source of protein. It will be necessary to the detection of fish mislabeling in order to protect the health and wellbeing of consumers, as well as their financial interests.

The use of electrophoretic techniques is widespread in the field of research pertaining to food items. Protein electrophoresis is a well-known technique that is utilized to ascertain the species of fish. Additionally, it is performed in order to validate the authenticity of marine products. The techniques of electrophoresis have a number of applications, including the identification of species and the research of proteomes in relation to the evaluation of product freshness, verifying the authenticity of food products, and the evaluation of processing conditions for both plant and animal products.

Ultimately, studies investigating the application of electrophoresis for fish species identification have demonstrated that the treatment of the fish, such as its state of being raw or cooked, might potentially influence the most suitable identification methods**.**

Conflict of Interest

The authors have no conflict of interest to declare.

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  - Title
  - Abstract (Should be between 100 and 350 words. References and abbreviations should be avoided)
  - Keywords (Minimum 3, Maximum 6 keywords)
  - Introduction
  - Materials and Methods
  - Results
  - Discussion (Can be combined with Results section if appropriate)
  - Conclusion
  - References
  - Table(s) with caption(s) (on appropriate location in the text)
  - Figure(s) with caption(s) (on appropriate location in the text)
  - and appendices (if any)

Manuscript Formatting

Use a 12-point Times New Roman font, including the references, table headings and figure captions, double-spaced and with 25 mm margins on all sides of A4 size paper throughout the manuscript. The text should be in single-column format.

- Each page must be numbered with Arabic numerals, and lines must be continuously numbered from the start to the end of the manuscript.
- Use italics for emphasis.
- Use only SI (international system) units.
- Use "dot" for decimal points.
- Use italics for species name.

References

Food Bulletin uses APA style (7th edition). Accordingly, authors must format their references as per the guidelines below. Please

ensure that each reference cited in the text is also presented in the reference list. Authors should always supply DOI or URL of the work cited if available.

In-text citation (Narrative):
…The results of Bliss (2022) support…
…Sönmez and Taştan (2020) indicated that…
…According to the method of Öztürk et al. (2021)…

In-text citation (In parenthesis):
…It was found to be isometric (Öztürk, 2018)…
…is highly susceptible to diseases (Doma & Craig, 2019)…
…have been studied (Kale et al., 2020)…

Two or more works in the same parenthesis:
…extremely toxic for the environment (Sönmez, 2018, 2019; Öztürk et al., 2020a; Kadak & Taştan, 2021)…

Citation in the reference list:
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Article:
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Tort, S. (1998). Stress and immune modulation in fish. *Developmental & Comparative Immunology*, *35*(12), 1366-1375. https://doi.org/10.1016/j.dci.2011.07.002

Kasumyan, A. O., & Døving, K. B. (2003). Taste preferences in fishes. *Fish and Fisheries*, *4*(4), 289-347. https://doi.org/10.1046/j.1467-2979.2003.00121.x

Özçelik, H., Taştan, Y., Terzi, E., & Sönmez, A. Y. (2020). Use of onion (*Allium cepa*) and garlic (*Allium sativum*) wastes for the prevention of fungal disease (*Saprolegnia parasitica*) on eggs of rainbow trout (*Oncorhynchus mykiss*). *Journal of Fish Diseases*, *43*(10), 1325-1330. https://doi.org/10.1111/jfd.13229

Article by DOI (early access):
Salem, M. O. A., Salem, T. A., Yürüten Özdemir, K., Sönmez, A. Y., Bilen, S., & Güney, K. (2021). Antioxidant enzyme activities and immune responses in rainbow trout (*Onchorhynchus mykiss*) juveniles fed diets supplemented with dandelion (*Taraxacum officinalis*) and lichen (*Usnea barbata*) extracts. *Fish Physiology and Biochemistry*. https://doi.org/10.1007/s10695-021-00962-5

Book:
Lastname, N., Lastname, M., & Lastname, O. (Year). *Title of the work*. Publisher.

Oidtmann, K., Xao, Q., & Lloyd, A. S. (2018). *The food need by the year 2050*. Elsevier.

Book Chapter:
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Pickering, A. D. (1993). Growth and stress in fish production. In G. A. E. Gall & H. Chen (Eds.), *Genetics in Aquaculture* (pp. 51-63). Elsevier. https://doi.org/10.1016/b978-0-444-81527-9.50010-5

Dissertation or Thesis:
Lastname, N. (Year). *Title of dissertation/thesis* (Doctoral dissertation/Master's thesis, Name of Institution).

Sönmez, A. Y. (2011). *Karasu ırmağında ağır metal kirliliğinin belirlenmesi ve bulanık mantıkla değerlendirilmesi* (Doctoral dissertation, Atatürk University).

Taştan, Y. (2018). *Tatlısu kerevitindeki (Astacus leptodactylus) siyah solungaç hastalığı etkeni mantar Fusarium oxysporum'un PCR yöntemi ile teşhisi* (Master's thesis, Akdeniz University).

Conference Proceedings:
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Ken, A., & Kumar, S. (2020). *A new statistical model for fuzzy logic evaluation*. 3rd International Congress on Statistics. İstanbul.

Institution Publication:
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FAO. (2020). *Fishery and aquaculture statistics 2018*. http://www.fao.org/3/cb1213t/CB1213T.pdf

Internet Source:
Lastname, N. (Year). *Title of the work*. Retrieved May 15, 2020, from URL

Perreault, L. (2019). *The future of agriculture: Polyculture*. Retrieved January 12, 2020, from https://www.agriculture.com

Table(s)
Tables, numbered in Arabic, should be in separate pages with a short descriptive title at the top. Place footnotes to tables below the table body and indicate them with superscript lowercase letters (or asterisks for significance values and other statistical data).

Figure(s)
All illustrations should be labelled as 'Figure' and numbered in consecutive Arabic numbers, Figure 1, Figure 2 etc. in the text. If panels of a figure are labelled (a, b, etc.) use the same case when referring to these panels in the text. Figures are recommended to be in electronic formats such as PNG, JPEG, TIFF (min. 300 dpi). All figures or tables should be presented in the body of the text.

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  - Completeness and accuracy of the references have been checked
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