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Determination of fish quality parameters with low cost electronic nose

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ABSTRACT

In the present study, the odour changes of trout, sea bream and sea bass were measured and recorded with Arduino microprocessor compatible MQ3, MQ4, MQ5, MQ8, MQ9 and MQ135 sensors during a week of storage. The odour intensity measured by the sensors were compared with the microbiological and sensory data, and spoilage thresholds were obtained. An electronic nose box was developed, which could quickly detect the quality of 10 g of fish meat placed inside using an Arduino microprocessor. Total viable counts of all test samples defined by the developed box as "Fresh Fish" were found to be were found to be lower than the level of 3 log CFU/g. The results of the study demonstrated that the odour sensors controlled by Arduino microprocessor could be fast, easy and low-cost solutions for determining the food quality parameters.

1. Introduction

Fish, which has rich fatty acids (Roy et al., 2019) and other nutritional components (Özoğul et al., 2013), should be consumed without loss of quality. The increase in the world population and the fact that the effects of healthy nutrition on human health have become evident, have led to the production of fish such as trout, sea bream and sea bass under culture conditions.

Food poisoning due to microbiological contamination causes the death of more than 400,000 individuals each year (WHO, 2020). Enzymatic reactions cause a series of negative consequences affecting the quality after the death of the fish. Changes that occur as a result of protein breakdown, and the changes in free fatty acid and increases in microbial loads make negative contributions to the quality of fish meat. Therefore, modern food technology conducts many physical, chemical, microbiological and sensory studies to determine the fish quality. Microbial spoilage is known to be the very important factor (Haute et al., 2016; Özoğul et al., 2013; Roco et al., 2018; Yavuzer et al., 2020) that spoils fish meat among these reasons for spoilage. Fish meat is in a sterile state while the fish is alive but the death of the fish causes the release of microorganisms such as *Pseudomonas oryzihabitans* and *Enterobacter cloacae* in the intestine, spread into the muscle and meet potential disruptors which leads to increase in microbial load in the fish during storage (Yavuzer, 2020). Some members of the microorganisms named the specific spoilage organisms, are destructive microorganisms and lead to the emergence of intense off-odour and flavor associated with the spoilage of fishery products (Gram & Dalgaard, 2002; Singh et al., 2016). The existence of human pathogens and the production of biogenic amine mainly histamine by bacteria make it crucial for seafood safety (Sheng & Wang, 2020). Based on this microbial increase, it is known that the spoilage of fish muscle components causes bad taste and odour, the production of polysaccharide causes the formation of mucus, and the production of CO_2 from the amino acids and often from carbohydrates causes the formation of gas (Gram & Huss, 1996).

The first process performed by any quality control authority before the food is consumed or processed is to smell the product. This applies to industrial and commercial procedures as well as individual consumer behaviors. Nonetheless, human senses often fail to function with full performance due to microbial, environmental or other reasons (Topaloğlu et al., 2020; Sakallı et al., 2020; Postma et al., 2020). Thus, scientists have conducted various studies on electronic noses, which operate similar to the human senses and provide standard results without being affected by the factors mentioned above (Majchrzak et al., 2021; Sarno et al., 2020; Tozlu et al., 2021; Silvello & Alcarde, 2020; Zhan et al., 2020; Zhu et al., 2020; Wang et al., 2020). Additionally, the ability to use minor apparatuses to solve major problems is an intended feature in terms of prototyping the devices (Yavuzer, 2018). Considering that the basic requirement in the electronic applications of the industrial field is the applicability in different platforms, easy coding and the ability to connect cheap sensors, the first device that comes into mind is Arduino.

Ardunio, which is an open-source microcontroller platform developed for the use of people from different fields, is a quite popular device that could perform simultaneous tasks with additional equipment and enable the desired prototype to be created. By using Arduino, signals coming from various sensors could be read, automations, where sensors

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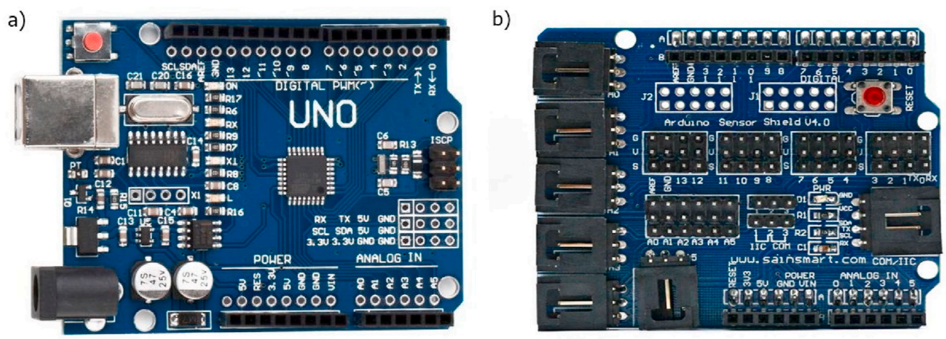

Fig. 1. Arduino Uno R3 microcontroller (a) and Arduino Uno Sensor Shield (b).

Table 1
The sensors used in the study and the gases they measure.

Sensor	Measurement
MQ3	Detects the presence of alcohol gas at an appropriate range of concentrations between 0.04 and 4 mg/L.
MQ4	Detects the presence of methane (CNG) natural gas between 300 ppm and 10000 ppm.
MQ5	Isobutane and propane detection between 200 ppm and 10000 ppm.
MQ8	It is used for the detection of 10–10000 ppm Hydrogen (H2) gas. It shows low sensitivity for detecting alcohol, LPG and cooking gases as well as hydrogen gas.
MQ9	Detects flammable gas concentration in the range of 100–10.000 ppm and carbon monoxide gas in the range of 10–10.000 ppm.
MQ135	Detects the amount of ammonia (10–300 ppm), alcohol vapor (10–300 ppm), benzene (10–1000 ppm)

work in connection with each other, could be developed or different motors could be operated. In addition to its wide scope of use, one of the most important features of Arduino is that, it has cheap sensors and microcontroller cards.

In our previous study (Yavuzer, 2020), we obtained a database by determining the voltage, color and liquid level using the Arduino microcontroller, and the change of odour by using MQ135. As a continuation of that study, it was planned to develop an electronic nose box for trout, sea bream and sea bass, with odour sensors working faster in the present study. Sensory scores and microbiological data obtained by experienced panelists during the storage of fish enabled the unintended thresholds of changes in fish quality to be interpreted with the data obtained by Arduino.

2. Material and methods

2.1. Electronics components used in device

In this study, an Arduino Uno R3 microcontroller board using AT mega328 microprocessor was used. Arduino Uno has 14 digital input/output pins. Six of them can be used as PWM (Pulse-width modulation) output. It also has 6 analog inputs, one 16 MHz crystal oscillator, USB connection, power jack (2.1 mm), ICSP (In-Circuit Serial Programming) header and reset button. Fig. 1a shows the Arduino Uno R3 used in this study.

Arduino Uno Sensor Shield was used to read all sensors together and simultaneously. Fig. 1b shows the Arduino Uno Sensor Shield. All input and output pins on Arduino Uno Sensor Shield are transformed into 3-pins in the order of DATA, VCC and GND and distributed on the board. In this way, all kinds of sensors, servos, relays, etc. can be easily attached to the card.

Table 1 shows the characteristics of the MQ sensors used in the study. The MQ-3 alcohol sensor is a gas sensor that outputs analog voltage while detecting the presence of alcohol gas at an appropriate range of concentrations between 0.04 mg/L and 4 mg/L. MQ-4 methane gas sensor is a sensor that can measure between 300 ppm and 10000 ppm

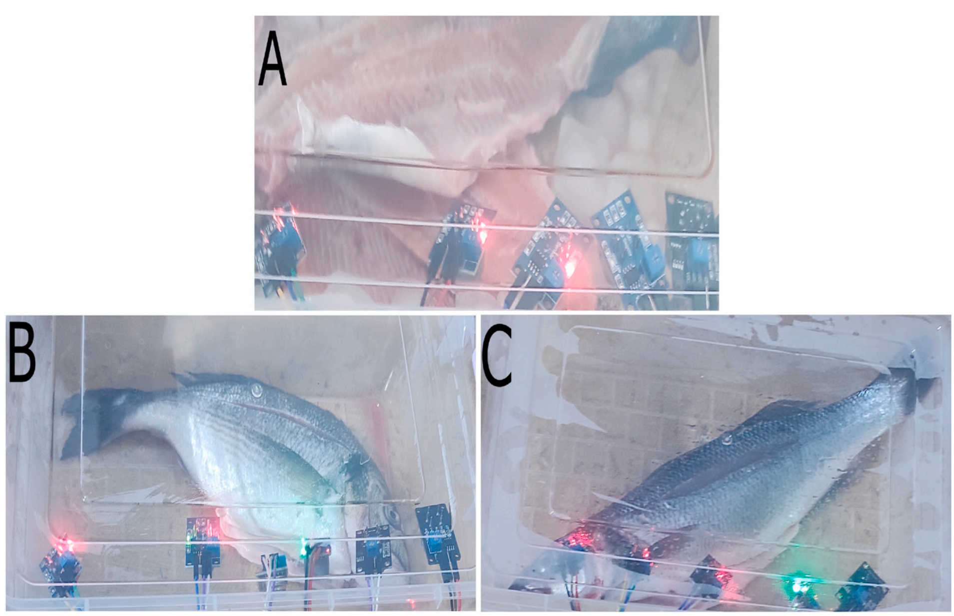

Fig. 2. Determination of odour values of fish with sensors. A: Rainbow trout, B: Sea Bream, C: Sea Bass.

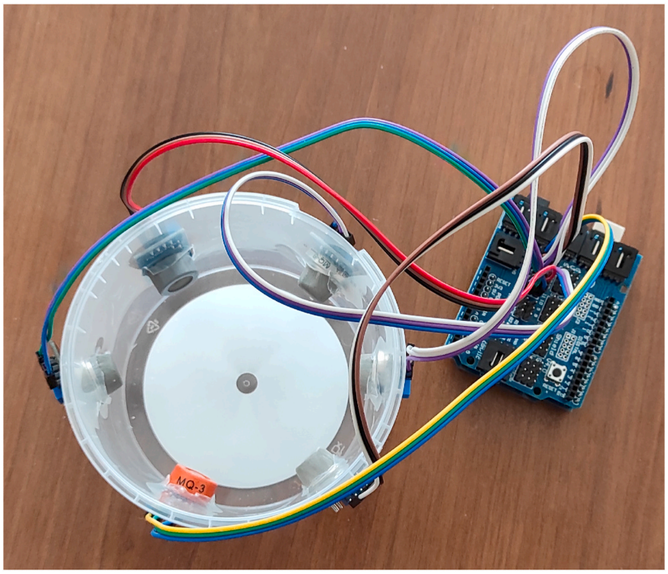

Fig. 3. Electronic nose box construction with MQ sensors.

and detects the presence of methane (CNG) natural gas. Like other MQ sensors, this sensor outputs analog voltage according to the density of the gas. The MQ-5 sensor detects isobutane and propane at 300 ppm and 10.000 ppm concentrations. The MQ8 sensor has high sensitivity to hydrogen but low sensitivity to alcohol vapor, LPG and smoke emitted from cooked food. The MQ9 gas sensor is a fast sensor operating at 5 V voltage, like other MQ sensors detecting the presence of 100 to 10,000 ppm flammable gas concentration and 10 to 10,000 ppm carbon monoxide (CO) in the environment. MQ135 is a gas sensor that calculates the ambient air quality by measuring the amount of ammonia, alcohol vapor, benzene, smoke and carbon dioxide gases.

2.2. Fish material

Rainbow trout (*Oncorhynchus mykiss* 350 ± 10g) used in the study was obtained from a trout production facility (EZG Corp. Hirfanlı Dam/Turkey). Sea bream (*Sparus aurata* 480 ± 12g) and sea bass (*Dicentrarchus labrax* 430 ± 22g) were purchased from the local fish market (FV Aquaculture Corp.) in Kırşehir, Turkey. Sea bream and sea bass were delivered to the laboratory in ice within 8 h of harvesting. The fish were stored in polystyrene boxes without ice at 3 ± 1 °C and selected randomly (just by reaching into the box without looking) on analyse days. All analyses were performed daily during storage.

2.3. Determination of odour levels of fish with MQ sensors

In the study, lidded plastic food containers were used to determine odour levels by sensors. Randomly selected fish were transferred to these containers and sensor readings were recorded. Determination of odour values of fish with sensors are given in Fig. 2.

2.4. Electronic nose box construction with MQ sensors

By determining the odour thresholds of whole fish during storage, a box was made to quickly detect the odour of smaller amounts of meat. For this, the plastic food storage box with lid was cut to pass the sensor heads from the sides. After the 10 g of fish meat sample was placed in the box, the measurements were made by closing the lid. The picture of the electronic nose box is given in Fig. 3.

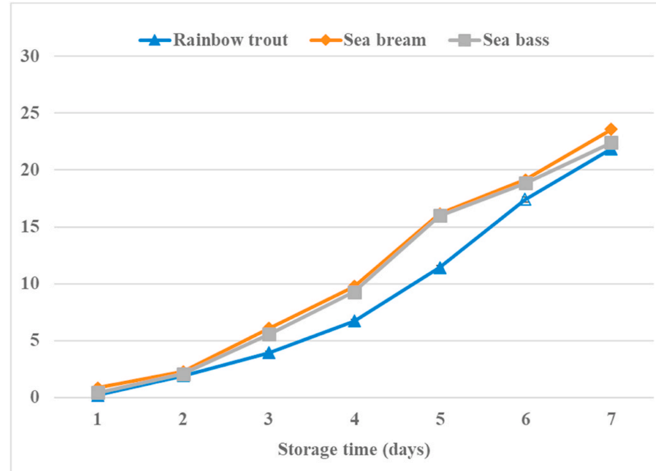

Fig. 4. Sensory changes in trout, sea bream and sea bass during storage 3 ± 1 °C.

2.5. Microbiological analysis

Triplicate samples were taken for total viable count (TVC). Ninety ml of ml of sterile Ringer solution (1/4 strength) was mixed with 10 g of fish muscle and then Stomached (Masticator Nr S18/420, IUL Instruments, Barcelona, Spain) for 3 min. More decimal dilutions were made, and then 0.1 ml of each dilution was pipetted onto the surface of plate count agar (Fluka 70152, Steinheim, Switzerland) plates in triplicate. After that plates were incubated for 2 d at 30 °C.

2.6. Sensory analyzes

Sensory analyzes of fish was made according to the method given by (Özoğul et al., 2017). The skin's brightness, mucus and scales, and the texture, water, odour, color, brightness and disintegration of the meat were scored by the panelists. In the scoring, 0 indicates the highest quality, while 3 indicates the worst quality. Analyzes were performed daily by 2 food engineers, 2 seafood engineers, 1 electronic engineer and 2 chef cook who were previously experienced in the sensory analysis of fisheries.

2.7. Statistical analyses

SPSS 22 version software (Chicago, Illinois, USA) used for one-way variance analysis (ANOVA) and Duncan's Multiple Range Test. Calculations were done in triplicate and comparisons at a p-value of <0.05 were carried out to point out significant differences.

3. Results and discussion

Fig. 4 demonstrates the sensory changes that occurred in trout, sea bream and sea bass during seven days of storage. The sensory evaluation score of trout was better than sea bream and sea bass from the beginning of storage to the end of storage. Meral et al. (2019), reported that trout fillets were sensually acceptable under cold storage conditions until the 4th day of storage and also Parlapani et al. (2014) reported the sensory shelf life of sea bream as 8 days at 5 °C, and 2 days at 15 °C under modified atmosphere packaging. Additionally, it has been reported in different studies that the sensory shelf life of sea bass is 3 days when stored at 5 °C, and 14 days at 1.8 °C (Chang et al., 1998; Çaklı et al., 2006). In the present study, fish were stored in polystyrene boxes without ice. While sensory rejection for trout occurred on the 6th day of storage, sea bream and sea bass were rejected on the 5th day of storage.

Thiobarbituric acid-reactive substances (TBARS) and peroxide value

Table 2
Changes in the TVC (log CFU/g) of fish during storage.

Days	Rainbow trout $\bar{x}\pm Sd$	Sea bream $\bar{x}\pm Sd$	Sea bass $\bar{x}\pm Sd$
1	1.50 ± 0.02^{cG}	2.14 ± 0.03^{aG}	1.96 ± 0.06^{bG}
2	2.08 ± 0.07^{cF}	3.03 ± 0.04^{aF}	2.94 ± 0.07^{bF}
3	3.87 ± 0.03^{cE}	4.12 ± 0.16^{aE}	3.96 ± 0.04^{bE}
4	4.21 ± 0.08^{cD}	5.45 ± 0.07^{aD}	5.29 ± 0.10^{bD}
5	5.56 ± 0.09^{cC}	7.12 ± 0.12^{aC}	7.09 ± 0.13^{bC}
6	7.43 ± 0.18^{cB}	9.21 ± 0.13^{bB}	9.48 ± 0.21^{aB}
7	9.11 ± 0.14^{cA}	9.53 ± 0.17^{bA}	9.77 ± 0.15^{aA}

Different letters (a – c) in the same column and different letters (A – G) in the same row show significant differences ($p < 0.05$).

Table 3
Changes in the odour values of trout given by Arduino microcontroller during storage.

Days	MQ3 $\bar{x}\pm Sd$	MQ4 $\bar{x}\pm Sd$	MQ5 $\bar{x}\pm Sd$	MQ8 $\bar{x}\pm Sd$	MQ9 $\bar{x}\pm Sd$	MQ135 $\bar{x}\pm Sd$
1	186 ± 1.06^{eG}	352 ± 1.23^{bG}	426 ± 3.97^{aG}	350 ± 1.03^{cG}	282 ± 0.71^{dG}	148 ± 0.48^{fG}
2	220 ± 0.84^{eF}	480 ± 0.79^{aF}	463 ± 0.70^{bF}	446 ± 1.62^{dF}	450 ± 0.99^{cF}	187 ± 0.42^{fE}
3	253 ± 0.67^{eE}	525 ± 0.74^{bE}	539 ± 1.34^{aE}	510 ± 1.42^{dE}	518 ± 1.84^{cE}	174 ± 0.48^{fF}
4	275 ± 0.91^{dD}	534 ± 1.19^{cD}	541 ± 0.73^{bD}	534 ± 1.22^{cD}	544 ± 1.03^{aD}	193 ± 1.03^{eD}
5	279 ± 0.52^{eC}	565 ± 1.03^{bC}	575 ± 1.88^{aC}	558 ± 2.14^{dC}	561 ± 1.26^{cC}	232 ± 1.88^{fC}
6	296 ± 0.87^{eB}	574 ± 1.31^{cB}	588 ± 2.17^{aB}	572 ± 0.42^{dB}	577 ± 3.86^{bB}	245 ± 0.52^{fB}
7	325 ± 1.28^{eA}	582 ± 3.33^{dA}	606 ± 3.40^{aA}	586 ± 0.67^{cA}	591 ± 2.79^{bA}	288 ± 0.48^{fA}

Different letters (a – f) in the same column and different letters (A – G) in the same row show significant differences ($p < 0.05$).

Table 4
Changes in the odour values of sea bream given by Arduino microcontroller during storage.

Days	MQ3 $\bar{x}\pm Sd$	MQ4 $\bar{x}\pm Sd$	MQ5 $\bar{x}\pm Sd$	MQ8 $\bar{x}\pm Sd$	MQ9 $\bar{x}\pm Sd$	MQ135 $\bar{x}\pm Sd$
1	318 ± 2.79^{eF}	466 ± 1.42^{cG}	477 ± 1.91^{bG}	458 ± 0.52^{dF}	482 ± 0.99^{aE}	194 ± 0.84^{fF}
2	325 ± 0.92^{eE}	474 ± 1.32^{bF}	489 ± 1.60^{aF}	459 ± 1.03^{cF}	445 ± 1.25^{dF}	204 ± 0.53^{fE}
3	304 ± 1.42^{eG}	519 ± 1.78^{bE}	532 ± 2.16^{aE}	491 ± 0.92^{cE}	429 ± 2.53^{dG}	185 ± 0.42^{fG}
4	342 ± 1.32^{eD}	532 ± 2.16^{bD}	544 ± 1.37^{aD}	515 ± 2.21^{cD}	509 ± 1.60^{dD}	215 ± 0.42^{fD}
5	388 ± 1.40^{dC}	561 ± 1.23^{bB}	557 ± 1.35^{cC}	556 ± 1.93^{cC}	564 ± 1.81^{aC}	225 ± 1.83^{eC}
6	407 ± 0.92^{eB}	551 ± 0.82^{dC}	569 ± 0.99^{cB}	603 ± 2.02^{aB}	587 ± 1.89^{bB}	280 ± 0.97^{fB}
7	417 ± 1.83^{eA}	607 ± 1.08^{cA}	616 ± 1.43^{aA}	611 ± 0.53^{bA}	604 ± 0.74^{dA}	309 ± 0.48^{fA}

Different letters (a – f) in the same column and different letters (A – G) in the same row show significant differences ($p < 0.05$).

(PV) are important parameters in determining lipid oxidation (Sallam, 2007) and chemical reactions are generally responsible for the initial loss of freshness, while microbial activity is responsible for the obvious deterioration (Gram & Huss, 1996; Sallam, 2007). Table 2 presents the changes in the total viable counts (TVC) during the cold storage of rainbow trout, sea bream and sea bass. Initial TVC was found to be 1.50, 2.14 and 1.96 log CFU/g for rainbow trout, sea bream and sea bass respectively. When the TVC values were examined together with the sensory analysis scores, it was observed that the sensory rejections started when the microbial load reached above the level of 7 log CFU/g on the 5th day of storage in the bream and sea bass groups, and on the 6th day of storage in the trout group.

Table 5
Changes in the odour values of sea bass given by Arduino microcontroller during storage.

Days	MQ3 $\bar{x}\pm Sd$	MQ4 $\bar{x}\pm Sd$	MQ5 $\bar{x}\pm Sd$	MQ8 $\bar{x}\pm Sd$	MQ9 $\bar{x}\pm Sd$	MQ135 $\bar{x}\pm Sd$
1	312 ± 1.32^{eG}	480 ± 0.88^{cF}	459 ± 2.84^{dF}	492 ± 0.42^{aG}	490 ± 0.84^{bE}	172 ± 0.52^{fG}
2	316 ± 0.48^{eF}	486 ± 1.08^{bE}	455 ± 0.82^{dG}	491 ± 0.82^{aF}	476 ± 1.83^{cF}	190 ± 1.49^{fD}
3	347 ± 1.06^{eE}	491 ± 0.52^{cD}	504 ± 1.42^{bcE}	506 ± 0.92^{aE}	477 ± 0.48^{dF}	180 ± 0.52^{fF}
4	364 ± 1.60^{eD}	517 ± 1.42^{dC}	541 ± 2.33^{bD}	529 ± 2.59^{cD}	553 ± 2.15^{aD}	184 ± 1.29^{fE}
5	380 ± 0.88^{dC}	567 ± 1.81^{bcB}	567 ± 1.32^{bcC}	583 ± 2.37^{aC}	569 ± 1.71^{bC}	235 ± 1.45^{eC}
6	412 ± 0.48^{eB}	616 ± 1.63^{bA}	582 ± 1.64^{dB}	620 ± 0.48^{aA}	611 ± 0.82^{cB}	249 ± 1.26^{fB}
7	424 ± 0.94^{dA}	616 ± 1.55^{bA}	611 ± 1.16^{cA}	617 ± 2.33^{bB}	625 ± 1.55^{aA}	275 ± 1.90^{eA}

Different letters (a – f) in the same column and different letters (A – G) in the same row show significant differences ($p < 0.05$).

The values measured by Arduino microcontroller for trout, sea bream and bass are presented in Table 3, Table 4 and Table 5, respectively. The values measured by the sensors increased regularly during storage. With a price tag of $1 in the robotic markets, the MQ-3 sensor is a sensor, which is used to detect the presence of alcohol gas in a range of concentrations suitable for making alcohol meters between 0.04 mg/L and 4 mg/L. Similar to the other MQ sensors, this sensor outputs analog voltage according to the density of the gas. The initial MQ3 sensor value of 186 ± 1.06 for trout increased to 296 ± 0.87 on the 6th day of storage, which was the point of sensory rejection. The value of 325 ± 1.28 measured by the MQ3 sensor for the trout on the last day of storage was very close to the initial MQ3 value of the sea bream and sea bass. MQ3 sensor value of trout was different from the sea bream and sea bass. The differences in this value may be due to the marine or freshwater origin of the fish used. On the other hand, it was observed that the microbial and sensory parameters reached undesired levels when sea bream and sea bass exceeded the limit of <350 in terms of MQ3 data.

In contrast to the readings of MQ3, which were different in the freshwater and saltwater fish, the MQ4 sensor analysis achieved a definitive standard in all three groups. At the beginning of storage, the sensor readings for trout, sea bream and sea bass were 352 ± 1.23, 466 ± 1.42 and 480 ± 0.88, respectively. On the 5th day of storage, when the TVC and sensory qualities decreased in the trout, it was found as 565 ± 1, and the TVC value of the trout was 5.56 log CFU/g. Similarly, the level of MQ4 sensor exceeded 561 ± 1.23 and 567 ± 1.32 on the 5th day of storage for sea bream and sea bass, respectively. The TVC values for sea bream and sea bass on the 5th day of storage were 7.12 log CFU/g and 7.09 log CFU/g, respectively. When the sensory and microbiological data were evaluated together with the readings of the sensor, the spoilage threshold for all three species analyzed in the study was found to be ≥ 550 for the MQ4.

The reading values by MQ5 in the first two days of storage, when the microbial and sensory data of all groups were at the highest level, were found as <500. The MQ5 level was above 550 for all groups on the 5th day of storage, when the fish exhibited the undesired quality characteristics. On the basis of changes in intense odour and sensory scores due to microbiological increase the value lower than 480 indicated very good levels for trout, sea bream and sea bass in terms of MQ5 and MQ4.

For MQ8 and MQ9, the value of ≤ 500 in the trout and sea bass groups indicated the freshness in the first two days of storage, while the sea beam group exceeded the value of 500 on the 3rd day of storage. The sensor level exceeded the 550 threshold on the 5th day of storage in all groups. After this day of storage, the MQ8 and MQ9 levels continued to increase significantly ($p < 0.05$) in all groups. The highest level for MQ8

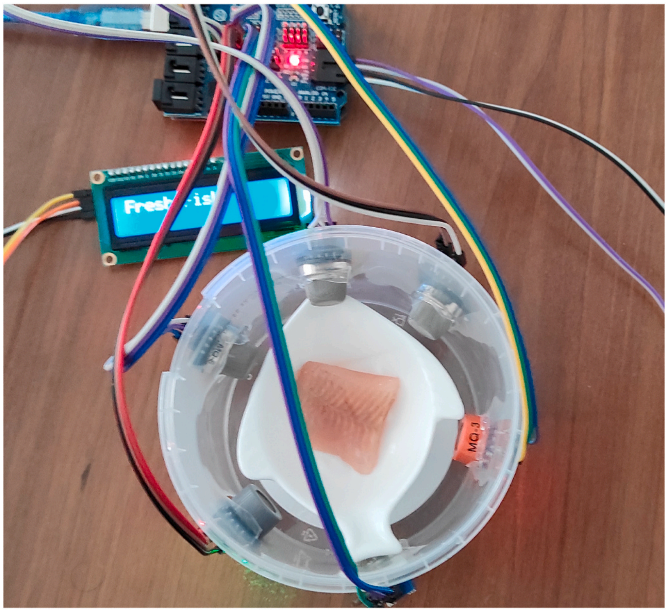

Fig. 5. Electronic nose box used to determine the odour changes of 10 gr fish.

was found as 620 ± 0.48 in the sea bass group on the 6th day of storage, while the highest level in MQ9 was 625 ± 1.55 in the sea bass group on the 7th day of storage again. A value of >560 measured by MQ8 and MQ9 signaled sensory rejection, and stinking fish for all groups.

MQ135 is a sensor that could detect the changes in the air such as ammonia, carbon dioxide, smoke and malodor (Sai et al., 2019); and it shows successful results in identifying the malodor (Farmanesh et al., 2019). The critical value measured by the MQ135 level was ≤220 for the fish that were analyzed in the study. It was determined that all groups, where this value was exceeded, were in undesirable stages in sensory and microbiological terms. In our previous study (Yavuzer, 2020), MQ135 levels of trout recorded during storage on ice were found to be 112 at the beginning of storage, and 291 on the eighteenth day of storage. In the present study, the MQ135 odour value was determined to be 288 ± 0.48 on the seventh day of cold storage. Among the sensors analyzed in the study, the sensor with the highest reading value variability was MQ135. All other sensors made rapid and stable readings when they entered the environment of the fish; however, it took a while for the value read by the MQ135 sensor to stabilize.

A program was developed to operate on Arduino by interpreting the relationship between the spoilage thresholds of fish, and the values measured by MQ sensors. Samples were obtained from the 10 g of fish meat in the range, and the sensor readings were performed for the samples using the electronic nose box presented in Fig. 5. The program was set to display "undesired quality" on the screen when the MQ sensor reading was >400, "fresh fish" when the readings of MQ4 and MQ5 were <490, and "undesired quality" when the readings of MQ4, MQ5, MQ8 and MQ9 were >550 in the fish samples that were placed in the box. The MQ135 sensor stabilizes later than other sensors and can rise above the critical threshold of 220 when it first starts. Therefore, a period of 2 min was allowed for the MQ135 sensor reading to be considered. The TVC analysis that was carried out on the samples obtained from the fish samples, for which the "Fresh fish" was displayed on the screen by the electronic nose box, were found to be lower than the level of 3 log CFU/g. In the present study, the TVC value of all the samples, for which "undesired quality" warning was displayed on the screen, was greater than the level of 5.00 log CFU/g.

4. Conclusion

In this study, a new data set was developed for determining the qualities of three commercial species, which were trout, sea bream and sea bass, using low-cost sensors with quick reading capacities connected to the Arduino microcontroller. Critical thresholds were determined by interpreting the obtained data set and the sensory-microbiological changes of fish. The "Electronic nose box", which was presented in this study, was developed according to these thresholds. The stability of the results obtained using the recently developed electronic nose box compared to the previously determined quality thresholds will contribute to the determination of spoilage limits for various foods other than fish. In addition, the fact that the electronic nose box, whose total cost does not exceed $20, is capable of performing quick and stable readings ensures that the data of the study are accessible and applicable.

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