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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Evaluation of lettuce (*Lactuca sativa* L.) in aquaponic system in terms of food safety

Marul bitkisinin (*Lactuca sativa* L.) akuaponik sistemde gıda güvenliği açısından değerlendirilmesi

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Abstract: We determined the number of coliform bacteria, *Escherichia coli*, yeast, and molds that may occur in the system, and the quality of lettuce grown in aquaponics for consumers from sensory, colour, and texture points of view. The amount of yeast and mold in the plant growing medium (hydroton) and water was 4.67 log CFU/cm^2 and 2.25 log CFU/mL at the end of the six-week experiment, respectively. The number of coliform bacteria and *E. coli* in the growing medium and in the system water was found to be 2.57 log CFU/cm^2 and 3.46 log CFU/mL for coliform, 0.75 log CFU/cm^2 0.31 log CFU/mL for *E. coli*, respectively. Organisms that pose a risk to food safety, accumulate in the culture media. After the harvest, lettuce cultured in the aquaponic system (AP) was compared with the lettuce cultured in soil (SC). According to the results, AP lettuce was found to have darker colors (Lightness: 56.4 AP, 49.09 SC, $p<0.05$), harder (Hardness: 209.3 AP, 153.7 SC, $p<0.05$), and slightly appetizing (Sensory analysis overall liking: 8.4 AP, 7.7 SC) than SC. In conclution, aquaponic systems are much more preferable in terms of sensory quality and consumer preferences than soil-based production systems.

Keywords: Aquaponics, lettuce quality, coliform bacteria, *Escherichia coli,* yeast and molds

Öz: Bu çalışmada, akuaponik sistemde üretilen marul bitkisinin duyusal tüketicii tercihi, renk ve doku kalitesi açısından değerlendirilmiş ve sistemde oluşabilecek koliform bakteri, *Escherichia coli*, maya ve küf miktarları belirlenmiştir. Bitki yetiştirme ortamındaki (hidroton) ve sudaki maya-küf miktarları, altı haftalık deneyin sonunda sırasıyla 4,67 log KOB/cm^2 ve 2,25 log KOB/mL olarak tespit edilmiştir. Hidrotonda ve sistem suyundaki koliform bakteri miktarı sırasıyla 2,57 log KOB/cm^2 ve 3,46 log KOB/mL, *E. coli* miktarı ise sırasıyla 0,75 log KOB/cm^2 ve 0,31 log KOB/mL olarak bulunmuştur. Gıda güvenliği açısından risk oluşturan mikroorganizmalar yetiştiricilik ortamında birikebilmektedir. Akuaponik sistemde (AP) yetiştirilen marul hasat edildikten sonra topraklı tarımda (TT) üretilen marul ile karşılaştırılmıştır. Sonuçlara göre AP marulun renkleri TT'den daha koyu (Parlaklık: 56,4 AP, 49,09 TT, p<0,05), daha sert (Sertlik: 209,3 AP, 153,7 TT, p<0,05) ve duyusal analize göre daha iştah açıcı (Genel duyusal beğenisi: 8,4 AP, 7,7 TT) bulunmuştur. Sonuç olarak akuaponik sistemler duyusal kalite ve tüketici tercihleri açısından topraklı üretim sistemlerine göre daha çok tercih edilmiştir.

Anahtar kelimeler: Akuaponik, marul kalitesi, koliform bakteri, *Escherichia coli,* maya ve küf

INTRODUCTION

Aquaponics is a combination of recirculating aquaculture and soilless agriculture. These systems are more advantageous than traditional agricultural techniques in water consumption, land use, soil salinization, yield, plant growth rate, chemical fertilizer requirement, and pesticide and herbicide usage factors. Although aquaponics has gained a lot of popularity in recent years, there is a lack of information in the field of food safety as it is a newly developing system (Hollyer et al., 2009).

The aquaponic system is an ecosystem of fish, plants, and bacteria which include both autotrophic and heterotrophic bacteria. Bacteria are essential to maintaining an aquaponic ecosystem (Blancheton et al., 2013; Eck et al., 2019; Schmautz et al., 2017). The successful administration of aquaponics depends on the complex microbial ecosystem it contains. Thanks to this microbial ecosystem, mineralization of nutrients required for plant production and biological cleaning of water are provided. However, while some species of these microorganisms in the system are beneficial, others may be harmful to human health.

There is a health risk of soil-borne agricultural pests, bacteria, and fungi in traditional soil farming. Although the risk of pathogen in aquaponic systems is less than in conventional agriculture (Somerville et al., 2014), it still exists (Yavuzcan Yildiz et al., 2017; Willmon, 2018). In aquaponic systems, pathogenic bacteria (e.g. *E. coli*) can enter the system in various ways due to the soil used in the germination stage of the plants used, the water added to the system daily, fish feed (Petreska et al., 2013), the digestive system of the fish or the non-sterile environment of the system.

Leafy vegetables have been increasing in popularity in aquaponics in recent years due to their great nutritional value and ease of use due to being a ready-to-eat product. However, due to pathogen contamination, leafy vegetables have caused numerous foodborne disease outbreaks (Hilborn et al., 1999; Taylor et al., 2013). In recent years, the number of outbreaks linked to *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. has increased (Deering et al., 2012; Strawn et al., 2013), of course, detection methods and back-tracking procedures (Brashears et al., 1999; Abadias et al., 2012), which have largely developed after an outbreak, also contributed to the detection of this increase. Fresh fruit and vegetables are responsible for approximately 48% of foodborne outbreaks (Hoagland et al., 2018). Additionally, aggressive processing methods like heat processing, acid treatment, etc. can not be used on fresh vegetables like lettuce without quality loss. Therefore, reducing the risk of foodborne pathogen contamination before harvest or until it reaches the consumer is a critical step.

No food outbreak has been encountered yet from a product grown in aquaponic systems (Kasozi et al., 2021). However, since this risk is always present, it is essential to examine the microbiological risks in aquaponic systems and to set appropriate standards. The risk to human health should be negligible.

There are several pieces of research on food safety in commercial aquaponic systems that aim to determine the levels of microorganisms in water and products (Chalmers, 2004; González-Alanis et al., 2011; Fox et al., 2022). *E. coli*, a coliform bacteria, is one of the most prominent. *E. coli* is a bacterium that is frequently employed as an indicator of fecal contamination and microbiological water quality in the formulation of regulatory standards based on human health.

In addition to bacterial load in terms of food safety, post-harvest quality is also an important parameter for the marketability of aquaponic products. Features such as color, size, appearance, and texture, which affect consumers' preferences, are strongly related to the sensory properties of lettuce (Schröder, 2003). According to Holmes et al. (2019), overall flavor and overall texture were stronger predictors of marketability than bitterness and crispness. This situation suggests that broader sensory categories, rather than narrower categories, may better capture human sensory perceptions of lettuce.

The purpose of this study was to determine the presence and amount of coliform bacteria, *E. coli*, yeast, and molds that may occur in the system when the wastewater of fish farming is given directly to the plant roots without passing through any disinfection system (UV, ozone, etc.) in a decoupled aquaponics system. Hence, we determine the texture, color, and sensory effects of the quality of lettuce grown in aquaponics for consumers and compared aquaponics lettuce with soil-grown lettuce.

MATERIAL AND METHODS

Growing conditions of the aquaponics system

This study was performed in decoupled aquaponics systems in Sapanca, Turkey. The aquaponic system is composed of a recirculating aquaculture system (RAS) and a hydroponic (HP) unit. There was four aquaponics and each AP include three fish tanks (400 L * 3), a sump (400 L), mechanical (Eheim Classic 1500 XL, Germany) and biological filtration units (500 L), a chiller (Teco TK-2000, Italy), and a blower (Aquaticlife PG-370, USA) in the RAS, while two plant beds (1.3 m^2 * 2) and a sump (400 L) were in the HP (Figure 1).

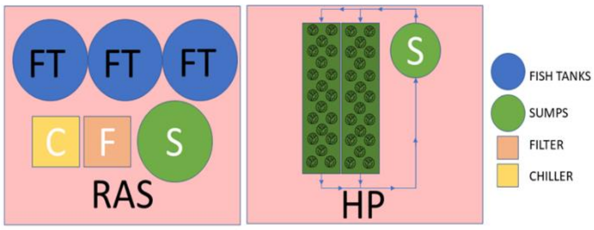

Figure 1. System design of the aquaponics

Lettuce (*Lactuca sativa* L. var. elmaria) seed germination was carried out in the soil. When they grew as 10-days-seedling, their roots were gently washed off with sytem water and transferred to the HP unit. The seedlings planted on pots with filled brand new hydrotons (Canna Aqua Clay Pebbles Hydroton 8-16 mm, Australia). The whole system had 240 cultivars (25 heads/m^2) and 432 rainbow trout (2.5 kg/m^3) (*Oncorhynchus mykiss* Walbaum 1792) juveniles. During the study, dissolved oxygen (9.94 mg/L), pH (8.3), temperature (15.7 °C), and electrical conductivity (257 µS/cm) parameters were followed daily and all values were kept at optimum conditions according to Somerville et al. (2014). The soil-cultured lettuce used in this study was obtained from a greenhouse in Istanbul. The harvest period of lettuce is 65 days.

Microbiological analysis

Water samples from the aquaponic system were collected in sterile glass bottles (1 L-Duran Shott bottles). Hydroton samples were collected under aseptic conditions using sterile gloves and immediately placed in sterile stomacher bags. Samples were kept in styrofoam box with ice gel packs during transport to the microbiology laboratory until bacterial enumeration on the same day. All microbiological analyzes were performed aseptically in a sterile laminar airflow cabin (CRYSTE, Korea).

The surface area of hydroton is determined by Nuevaespana and Matias (2022) and it was 450 m^2/m^3. Hydroton samples (n~10) were placed in a sterile bag and diluted with maximum recovery diluent (MRD) at a 1:10 ratio before being blended with a stomacher. The water and hydroton samples were diluted serially. Spread plating was done in triplicate for each dilution sample.

Total coliform and *Escherichia coli*

Total coliform and generic *Escherichia coli* counts were according to Feng et al. (2022). One milliliter of the sample was spread plated on VRB agar with MUG plates (VRB (Violet Red Bile Lactose)-MUG agar Merck 1.04030). All spread plates were incubated at 37 °C for 18-24 hours before counting typical pink colonies to determine the total coliform count. To define generic *Escherichia coli*, colonies that fluoresced under UV light were counted. The colonies were then recorded for analysis.

Yeast-Mold

The yeast and mold were counted using Dichloran Rose Bengal Chloramphenicol Agar (DRBC) (Merck, 1.00466). All spread plates were incubated for 5 days at 25°C (Tournas et al., 2022).

Sensory analysis

Sensory quality was determined as described by Martínez-Sánchez et al. (2011), by a ten-member trained panelist. Samples were evaluated by changes in visual quality, flavour, odour, texture, and browning of the leaf edge/surface. Samples of lettuce were properly washed with sterile Milli-Q water before sensory evaluation, dried with paper towels, and kept in the refrigerator (at 4 °C) in zipper-sealed plastic bags until analysis. Prior to the sensory panel, a 3-digit numerical code was randomly issued to each sample. Individual leaves that did not contain the innermost or outermost leaves made up the sample parts. Consumers were asked to score sensory quality using a 9-point hedonic scale, where 9 = excellent, 5 = limit of marketability and 1 = inedible.

Color analysis

The color measurements of aquaponics grown and soil culture lettuce samples were determined with the Konica Minolta Chromometer (model CR 400; Minolta, Osaka, Japan). After the calibration using a white reference tile (CR-A44; L* = 94.93, a* = −0.13, b* = 2.55, and C* = 2.55), the lightness (L* value), the color (a*: + a, red; – a, green, b*: + b, yellow; – b, blue) and Munsell Hue (H) (GY; Green Yellow), Munsell Value (V) and Munsell Chroma (C) were measured three times on the samples at 3 different locations (E: Edge, M: Middle, S: Stem) on each leaf (n=10). The results are presented as the mean ± SD for the triplicate samples (Gerdes and Santos Valdez, 1991).

Texture analysis

Texture profile analysis (TPA) on aquaponics grown and soil culture lettuce samples were determined by using a texture analyser (Brookfield CT3 Texture Analyzer, USA), equipped with a blade set probe, described by Back et al. (2014). Three stacked samples (3 by 3 cm) were placed onto the press holder, and a blade was moved down at 2 mm/s. TexturePro CT software (version 1.2, Brookfield Engineering Laboratories, Inc.) was used for the tabulation of TPA values (hardness, springiness, cohesiveness, chewiness, average peak load). The test had the following parameters: a pretest speed of 2 mm/s, a test speed of 1 mm/s, and a post-test speed of 1 mm/s. A measure of hardness was defined as the maximum force necessary to shear the samples. All experiments were performed three times, with independently-prepared samples (n=3) from three different parts of leaves (E: Edge, M: Middle, S: Stem) as in Figure 2.

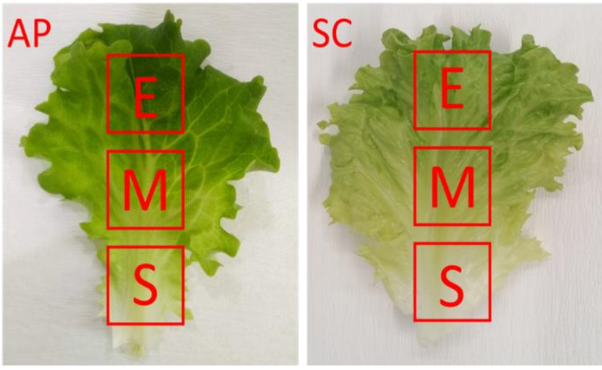

Figure 2. Different sampling locations on aquaponics grown (left) and soil culture lettuce (right) leaves (AP: Aquaponics grown lettuce, SC: Soil culture lettuce, E: Edge, M: Middle, S: Stem)

Statistical analysis

The data were analyzed using IBM Statistical Package for the Social Sciences 28 software (USA). All data are given as mean values ± standard deviation (SD). The hydroton and water samples were examined at two-week intervals for a total of 6 weeks. Significant differences between groups on yeast and mold data were determined by ANOVA (post-hoc Tukey and Duncan) test, on coliform bacteria was determined by a tailed-independent Student's T-test. To identify significant differences between aquaponics and soil-cultured lettuce samples in terms of sensory, color, and texture analyses, a tailed-independent Student's T-test analysis was used. A 95 percent level of confidence was used for all statistical analyses ($p < 0.05$).

RESULTS

Yeast and mold results in the first week, third week, and sixth week were determined as 3.31±0.8, 2.29±0.55, and 2.25±0.51 log CFU/ml in water samples and 2.41±0.25, 3.57±0.58, 4.68±0.63 CFU log/cm^2 in hydroton samples, respectively. While there was no difference between hydroton and water in the first week, there was a significant difference in the third and sixth weeks. Throughout the study, there is a decreasing trend (R^2 = 0.78) in water samples while an increasing trend (R^2 = 0.99) in hydroton samples (Figure 3).

Although the mean number of coliform bacteria was higher in water samples (3.46±0.65 log CFU/ml) compared to hydroton samples (2.57±1.53 log CFU/cm^2), a statistical difference was not detected between the groups (Figure 4).

The total *E. coli* count is given in Figure 5. Results show that growing media had more colonies than water. However, there were no significant differences between groups.

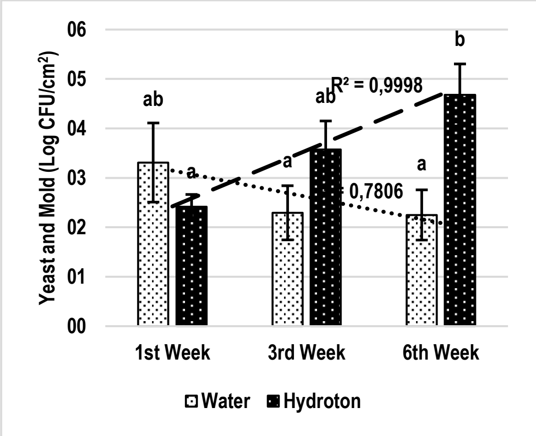

Figure 3. Mean Log count of yeast and mould colonies on water (log CFU/ml) and hydroton samples (log CFU/cm^2) (mean values ± SD, *n* = 4, Different letters on the top of SD bars indicate significant differences between mean values, Tukey's HSD multiple comparison method, *P* < 0.05)

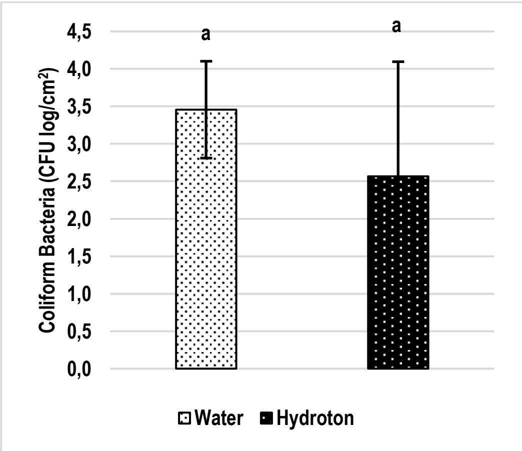

Figure 4. Coliform bacteria count of hydroton (log CFU/cm^2) and water samples (log CFU/ml) (mean values ± SD, *n* = 4, Different letters of the top on SD bars indicate significant differences between mean values, Student's T-Test, P < 0.05)

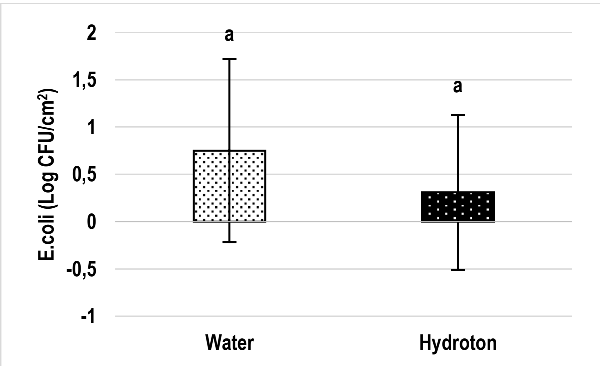

Figure 5. *E. coli* count on water (log CFU/ml) and hydroton (log CFU/cm^2) media at the end of the study

According to the overall liking of consumers, aquaponic lettuce has a mean value of 8.4 out of 10, while lettuce grown in soil has a mean value of 7. Lettuces that are cultivated in the aquaponics system had slightly higher quality than those cultivated in soil but the differences were not significant. The results from the sensory panel such as visual, flavour, texture, odour parameters are found as similar to overall liking results as given in Figure 6.

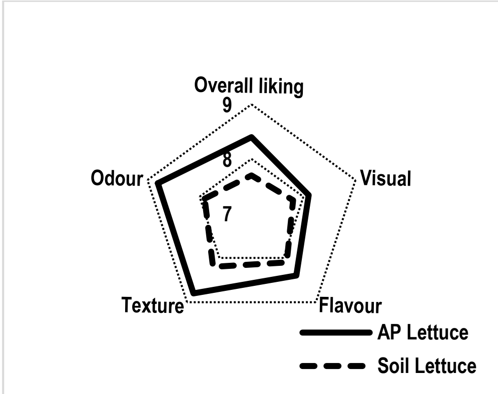

Figure 6. Sensory panel evaluations of aquaponics-grown lettuce and soil-grown lettuce (Mean ± standard deviation, AP: Aquaponics grown lettuce, SC: Soil culture lettuce, E: Edge, M: Middle, S: Stem)

The leaves of lettuce grown in aquaponics and lettuce grown in soil were examined in three regions (Edge, Middle, Stem) (Figure 2) and compared in terms of hardness, springiness, and cohesiveness (Figure 7).

The hardness value at the edge of AP (347.67 g) lettuce leaves was found to be statistically significantly higher than SC (261 g). The amount of hardness in the middle of the leaf was measured as 190.8 g for AP and 152.33 g for SC, while the amounts in the stem were measured as 89.5 g and 47.8 g. According to these results, it was determined that AP leaves had higher hardness values than SC. In addition, it was observed that the hardness values decreased from the edge of the leaves to the stem (Figure 7).

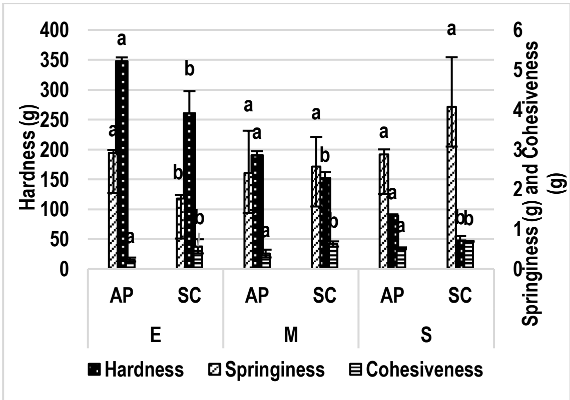

Figure 7. Hardness (left side), Springiness and Cohesiveness (right side) parameters of aquaponics grown lettuce and soil-grown lettuce (Mean ± standard deviation, AP: Aquaponics grown lettuce, SC: Soil culture lettuce, E: Edge, M: Middle S: Stem)

Color parameters such as L*, a*, b*, Munsell hue, value and chroma are given in Figure 8. AP lettuce varied from green-yellow and had a Munsell hue between 5.2-5.9. Conversely, SC lettuce was between 5.1 and 5.8.

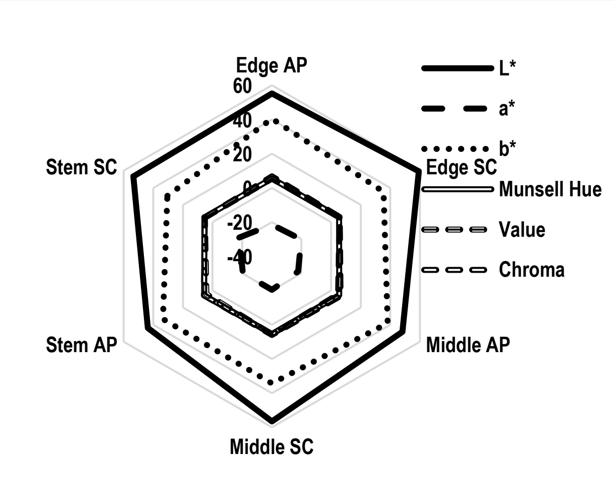

Figure 8. Color and texture parameters of aquaponics-grown lettuce and soil-grown lettuce (Mean ± standard deviation, AP: Aquaponics grown lettuce, SC: Soil culture lettuce, E: Edge, M: Middle S: Stem)

DISCUSSION

The microbial profiles and counts (yeast and mold, coliforms, *E. coli*) for soil cultured and aquaponics-grown lettuce were determined by the current study. Yeast, mold, and coliform bacteria were detected in all 480 samples taken from the water and hydroton of the aquaponics system. Our findings are consistent with Sirsat and Neal (2013), Moriarty et al., (2018), and Weller et al. (2020) who observed coliforms or yeast and mold in all samples collected regularly from aquaponic and hydroponic systems. In our study, there was more yeast and mold in the hydrotons where the plants are attached by their roots compared to the water in the system. This may be due to microorganisms in the root environment being dependent on root exudates (Khalil, 2018). There was no difference in coliform bacteria amount between hydroton and water samples. *E. coli* was found in 50% of the four aquaponic systems constructed. According to Sirsat and Neal (2013), soil culture lettuce contained 2 to 3.5 log CFU/g *E. coli*. The amount of yeast and mold in the water, which was 3.31 ± 0.8 log CFU/g in the first week, was similar to the hydroton results of 2.41 ± 0.25 log CFU/g. The yeast and mold results in the third and sixth weeks were 2.29 ± 0.55 log CFU/g and 3.57 ± 0.58 log cfu/g, respectively, while the yeast and mold values in the hydroton were 2.25 ± 0.8 log CFU/g and ± 0.8 log CFU/g, respectively. Similar results in fungal flora of aquaponic system water between 2.8-3.4 log CFU/mL were observed by Khalil (2018). Therefore, the amount of yeast and mold on the hydroton to which the roots of the plants are attached showed an increasing trend between the third and sixth weeks compared to the first week (R^2=0.99), and a decrease (R^2=0.78) was observed in the water as time passed. This is thought to be because hydrotons create a suitable environment for yeast and mold organisms, like as nitrifying bacteria need surface area to form colonies (Pedersen et al., 2017).

Sirsat and Neal (2013) made a comparative microbial analysis of lettuce cultured with different techniques such as conventional, organic, bagged, farmers' market, and aquaponics and found lettuce from farmers' markets contained 2 to 3.5 log CFU/g *E. coli*. Organic and conventional lettuce contained about 2 log CFU of *E. coli* per gram of lettuce. According to Tyson et al., 2012, the population level of the coliform is 2.2 ± 0.2 log CFU/mL in aquaponics. Our findings show that aquaponic production has lower levels of coliform, mold, and yeast contamination compared to other production systems (Sirsat and Neal, 2013), and these findings are in agreement with the results of previous studies (Tyson et al., 2012; Elumalai et al., 2017).

Market pricing and consumer choice for lettuce are determined by internal attributes such as functional nutritional value and texture, as well as external qualities like color and size (Mampholo et al., 2016). In this study aquaponics grown lettuce scored higher in terms of sensory properties than those grown in soil. Similar results for soilless red-leafed lettuce cultures were reported by Selma et al. (2012), however, there are contrasting data for green leaf lettuce in the same study. For the textural properties, Fontana et al. (2018) reported that the hydroponic lettuces were significantly better accepted in relation to organic samples. The sensory approval of lettuce grown in conventional, hydroponic, and organic growing systems were tested using the hedonic scale by Fávaro-Trindade et al. (2007), and similarly, the authors did not observe any significant differences. Aquaponics products could have an odour because of the off-flavors (Atique et al., 2022). However, consumers stated that the lettuce grown in aquaponics did not have a fishy odor, which may have been the reason why they were not preferred aquaponics products by consumers.

Physiological properties such as color, size and texture are known to be affected by the conditions and environment in which lettuce is grown (Lei and Engeseth, 2021). There is variation in our TPA results as some lettuce pieces contain lettuce hearts and others are much leafier as in Predmore et al. (2015). Ibrahim and Zuki (2012) found that lettuce grown hydroponically had a much higher tensile strength than lettuce grown aquaponic and then planted in the ground. This showed that lettuce crispness values were also higher when grown hydroponically. None of the measured force values were significantly different from the soil-grown lettuce samples. However, this may be due to the small sample size of *n* = 3 as in Schnabel et al. (2021). Also, Lei and Engeseth (2021) stated that when compared to lettuce grown in soil, lettuce grown hydroponically has softer leaves and tighter midrib, which may be due to lignin in the cell wall. In accordance with this our values show that leaf tissue became softer from S and E to M.

In our study, edge, middle, stem of lettuce samples from SC had a higher L* level than AP. In general AP groups had a greener color in leaves than SC lettuces, which could be related to concentrations of chlorophyll in the leaves. Soil and aquaponics nutrients could be the reason for color differentiation. According to Ibrahim and Zuki (2012), regardless of plant growing technique, there was no significant difference in plant color. Matthew T et al. (2011) compared the visual quality of different lettuce varieties grown under hydroponic, conventional and organic conditions, and no difference was detected between groups. Similar results were observed by Lei and Engeseth (2021) who compared hydroponics vs soil-grown lettuce. On the contrary, Fontana et al. (2018) stated that hydroponically grown lettuce was lighter in color compared to organic and conventional ones. Outer leaves of green cultivars have lower (more negative) a* values due to higher chlorophyll content and darker green hue, according to Ozgen and Sekerci (2011). Our study showed that AP had slightly higher a* value than SC. Color results show that it can be affected by environmental conditions and phytochemical processes such as secondary metabolites (Ozgen and Sekerci, 2011).

CONCLUSION

In conclusion, it has been determined that there is a risk of pathogens in the edible parts of the plants produced in aquaponic systems, the media used in the media bed technique poses a greater risk than the water, and the lettuce grown in aquaponics is preferred by the consumers because has stiffer leaves, darker green color and better quality for the consumer than the lettuce produced in traditional soil agriculture. Since organisms at risk accumulate in the culture media, it is necessary to compare the 'media bed technique' with the 'nutrient film technique' or 'deep water culture technique' in future studies. Increasing awareness of the state of scientific issues regarding indicator organisms in an aquaponic setting enables the consumer to make informed decisions. Thus, an increase in food safety in products produced through aquaponics is ensured. Determining the color, texture, and sensory profile of aquaponics products can be important tools in deciding under what conditions the crop will be grown and marketed. Food preferences are impacted by sensory perceptions. It is critical for producers to carry out research on this subject in order to determine which cultivar will be grown to meet market demands throughout the year. Studies on the quality and consumption risks of the products produced in the aquaponic system should be done more comprehensively.

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AUTHORSHIP CONTRIBUTIONS

Gökhan Tunçelli and Devrim Memiş: Conceptualization, methodology. Gökhan Tunçelli: Data curation, writing- original draft preparation. Gökhan Tunçelli and İdil Can Tunçelli: Visualization, investigation. Devrim Memiş: Supervision, project administration, resources, funding acquisition. Gökhan Tunçelli, İdil Can Tunçelli and Devrim Memiş: Writing-reviewing and editing.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

ETHICS APPROVAL

This study was approved by the local ethics committee for animal experiments of Istanbul University, Istanbul, Turkey (Approval No. 2019/10).

DATA AVAILABILITY

Not applicable.

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