

## MICROBIOLOGICAL QUALITY OF LETTUCE AND NUTRITIONAL COMPOSITION OF LETTUCE AND FISH IN AN AQUAPONIC SYSTEM

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### Abstract

Aquaponics is a system that integrates fish production with vegetable cultivation, resulting in a considerable reduction in water consumption and fertilizer use compared with traditional methods. However, as it is a relatively new technology, consumers still exhibit some degree of distrust regarding the quality and safety of foods produced in these systems. Taking this into account, the present study aimed to perform the microbiological and nutritional characterization of fish and vegetables produced in a Media-Based Technology (MBT) aquaponic system, in order to ensure the quality and safety of the foods produced. Thus, ten fish and ten plants cultivated in an MBT system were sampled. Microbiological analyses of lettuce and the determination of the proximate composition of lettuce and fish were conducted. It was observed that the proximate composition of fish reared in aquaponic systems did not differ from that of fish reared in conventional production systems, with moisture ( $78.81 \pm 0.822$ ), protein ( $12.614 \pm 1.143$ ), lipids ( $3.785 \pm 0.454$ ), and ash ( $1.558 \pm 0.265$ ). In addition, the vegetables produced in this system did not exhibit any type of contaminant that would preclude their commercialization and consumption. The following values were observed for lettuce: moisture ( $94.13 \pm 0.439$ ), protein ( $2.200 \pm 0.482$ ), lipids ( $2.492 \pm 0.428$ ), and ash ( $1.175 \pm 0.363$ ). Therefore, aquaponic systems demonstrate great potential for future food production.

**Keywords:** Aquaponics; *Lactuca sativa* L.; Tilapia

### Resumen

La acuaponía es un sistema que integra la producción piscícola con el cultivo de hortalizas, lo que se traduce en una reducción considerable del consumo de agua y del uso de fertilizantes en comparación con los métodos tradicionales. Sin embargo, al tratarse de una tecnología relativamente nueva, los consumidores siguen mostrando cierto recelo respecto a la calidad y la seguridad de los alimentos producidos en estos sistemas. Teniendo esto en cuenta, el presente estudio tuvo como objetivo realizar la caracterización microbiológica y nutricional de los peces y las hortalizas producidos en un sistema acuapónico de tecnología basada en medios (MBT), con el fin de garantizar la calidad y la seguridad de los alimentos producidos. Para ello, se tomaron muestras de diez peces y diez plantas cultivadas en un sistema MBT. Se realizaron análisis microbiológicos de la lechuga y se determinó la composición aproximada de la lechuga y el pescado. Se observó que la composición aproximada del pescado criado en sistemas acuapónicos no difería de la del pescado criado en sistemas de producción convencionales, con humedad ( $78,81 \pm 0,822$ ), proteínas ( $12,614 \pm 1,143$ ), lípidos ( $3,785 \pm 0,454$ ) y cenizas ( $1,558 \pm 0,265$ ). Además, las hortalizas producidas en este sistema no presentaban ningún tipo de contaminante que impidiera su comercialización y consumo. Se observaron los siguientes valores para la lechuga: humedad ( $94,13 \pm 0,439$ ), proteínas ( $2,200 \pm 0,482$ ), lípidos ( $2,492 \pm 0,428$ ) y cenizas ( $1,175 \pm 0,363$ ). Por lo tanto, los sistemas acuapónicos demuestran un gran potencial para la producción alimentaria futura.

Palabras clave: Acuaponía; *Lactuca sativa* L.; Tilapia

## Introduction

The growing global demand for food, combined with the impacts of climate change and urban expansion, has intensified pressure on production systems and available natural resources (Verdoliva *et al.*, 2021). In this context, ensuring the supply of safe and nutritious food has become one of the major contemporary challenges, particularly in light of United Nations projections indicating that the global population may reach 8.5 billion by 2030. This scenario reinforces the need to develop more efficient and sustainable production alternatives that are adapted to reducing the use of conventional inputs, such as water and fertilizers.

Among the promising approaches to increasing resource-use efficiency, integrated agriculture–aquaculture systems have stood out for enabling the reuse of water and nutrients, thereby reducing costs and environmental impacts. Aquaponics, in particular, has received increasing attention for integrating fish and plant cultivation in recirculating systems in which water is continuously treated and reused (Bailey & Ferrarezi, 2017). In this model, fish metabolic wastes are converted into nutrients assimilable by plants, promoting a low-waste production system (Monsees *et al.*, 2019).

Despite its benefits, microbiological safety in aquaponic systems remains a subject of debate. The presence of aquaculture effluents may represent a potential risk of vegetable contamination, especially when consumed raw (Sirakov *et al.*, 2016; Wielgosz *et al.*, 2017). Recent studies have highlighted the need for a better understanding of the routes of entry and persistence of pathogenic microorganisms in these systems, emphasizing scientific gaps related to contamination mechanisms and the conditions that favor their occurrence (Sawyer, 2021; Kasozi *et al.*, 2022).

Lettuce occupies a central position in these discussions, as it is one of the most widely consumed fresh vegetables and one of those most frequently associated with foodborne disease outbreaks (Cardinale *et al.*, 2015). In addition, it is the main plant species cultivated in aquaponic systems (Bailey, 2017). On the animal side, tilapia is the fish most commonly used in these systems (Krastanova *et al.*, 2022) and hosts a diverse microbiota (Banerjee, 2017; Hamom *et al.*, 2020), including microorganisms potentially pathogenic to humans and capable of contaminating foods consumed raw (Sawyer, 2021; Bereded *et al.*, 2022).

Beyond microbiological aspects, the quality of foods produced in integrated systems involves nutritional, sensory, and physiological factors, which determine their

acceptance and their contribution to food security. In vegetables such as lettuce, attributes including appearance, crispness, and nutritional composition are central to ensuring products suitable for consumption (Sutani *et al.*, 2021).

In light of these considerations, the present study aims to evaluate the nutritional quality of curly lettuce and fish produced in a Media Bed Technique (MBT) aquaponic system. This research seeks to expand understanding of the efficiency of this integrated production model, as well as its capacity to provide safe, nutritious, and environmentally sustainable foods, thereby contributing to the strengthening of more resilient food systems.

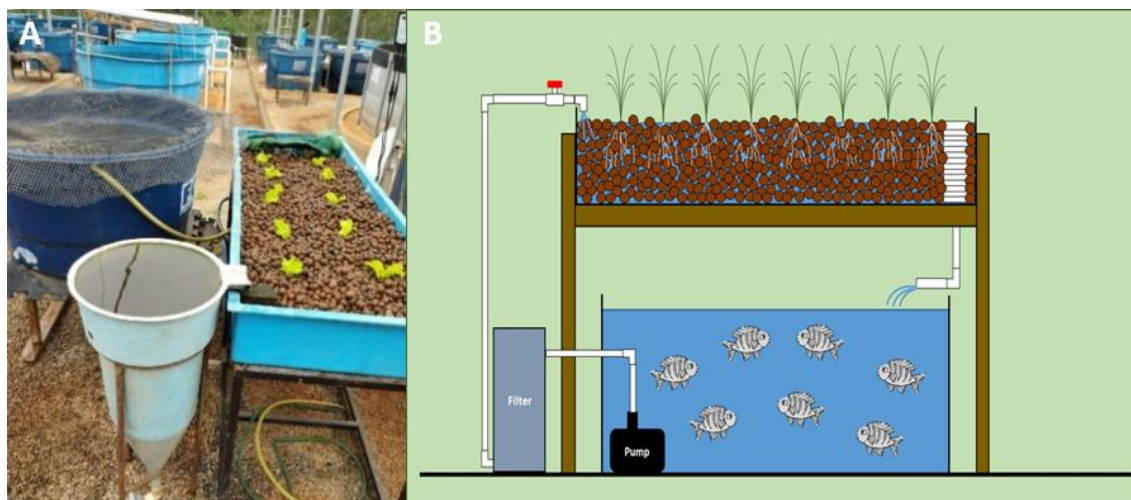
## Materials and methods

The study was approved by the Animal Use Ethics Committee (CEUA) of the Federal University of Lavras (UFLA), Minas Gerais, Brazil, under protocol no. 037/22. The research was conducted at the Fish Farming Sector of the Department of Animal Science, at the Pilot Fish Processing Plant, and at the Advanced Analysis Laboratory and the Microbiology Laboratory of the Department of Food Science (DCA).

The limnological characteristics of the water were measured weekly. Temperature was measured using a glass liquid thermometer, while pH, ammonia, and nitrite were determined using a Labcon® colorimetric kit. Water temperature in the fish-rearing tank of the aquaponic cultivation system was  $25 \pm 2$  °C, pH was 6.8, ammonia concentration was  $0.50 \pm 0.11$  ppm, and nitrite concentration was zero.

Lettuce and fish samples were produced in a Media Bed Technique (MBT) aquaponic system, also known as a grow bed or gravel-based cultivation environment. This system consists of cultivation in which plant biomass is supported by a substrate with continuous water flow supplied by the fish tank (Azad *et al.*, 2016; Ventura *et al.*, 2019) (Figure 1). Substrates with a wide particle-size distribution are used, allowing complete drainage while maintaining moisture at the roots, thereby enabling the nitrification process in symbiosis with nitrifying bacteria and nutrient absorption as the water flow reaches the plant roots (Magalhães *et al.*, 2022). One of the advantages of media-based hydroponic systems is that the substrates provide an adequate amount of surface area for the growth of nitrifying bacteria and for physical filtration, eliminating the need for a separate biofilter (Maucieri *et al.*, 2018).

Figure 1 – Media bed-type aquaponic system, A: MBT system; B: illustrative image of the grow bed of the MBT system.



Source: A: System of the Federal University of Lavras; B: (Beecher, 2021).

The system consisted of a 1,000-L plastic water tank used for fish rearing, a fiberglass cylinder with a conical bottom used as a settler, and a fiberglass grow bed. A bell siphon was installed in the grow bed to generate the tidal movement (automatic filling and draining at a predetermined level), and the entire internal space was filled with expanded clay, which served both to anchor the seedlings and as a biological filtration medium. Finally, the grow bed drained into a sump with a capacity of 100 L, where a submersible water pump was installed to return the water to the fish rearing tank and promote water oxygenation. To enhance water oxygenation efficiency, a Venturi-type aerator was developed using 25-mm PVC pipes and fittings.

After completion of the aquaponic system assembly, fish rearing activities were initiated. Although the maximum stocking capacity is approximately 40 kg of biomass, a density of 30 kg was adopted to ensure greater system stability and animal welfare. The fish were fed twice daily to apparent satiation with extruded feed containing 36% crude protein. After the biological

maturation phase, ten curly lettuce (*Lactuca sativa* L.) seedlings, acquired from a local agricultural supplier and originating from conventional soil cultivation, were introduced. The seedlings were transplanted at a spacing of  $25 \times 25$  cm, ensuring adequate light availability, nutrient supply, and space for proper vegetative development.

#### Experimental Sampling

Curly lettuce (*Lactuca sativa* L.) plants were harvested 45 days after transplanting into the MBT system. Harvesting was carried out in the early morning (Figure 2) in order to preserve the physiological integrity of the plants. Subsequently, the units were subjected to sanitization in a solution containing 100 ppm of active chlorine for 5 minutes, dried with paper towels, and individually packaged in properly identified polyethylene plastic containers. Thereafter, the samples were placed in plastic boxes made of the same material and stored under refrigeration at 4 °C until the analyses were performed.

Figure 2 – Lettuce harvested after 45 days.



For the collection of the ten specimens of red tilapia (Figure 3), the fish were first subjected to a 24-hour fasting period. They were then induced into deep anesthesia and euthanized by the administration of benzocaine at a concentration of  $300 \text{ mg}\cdot\text{L}^{-1}$ . After reaching complete insensibility, characterized by the

absence of opercular movements and muscular activity, spinal cord sectioning was performed, according to the method described by McFarland (1959) and cited by Ross and Ross (2008). Subsequently, the specimens were filleted, and the obtained samples were frozen at  $-18^\circ\text{C}$  until the analyses were conducted

Figure 3 – Tilapia specimens after slaughter and filleting.



### Analytical Methods

In the assessment of physicochemical quality, the following parameters were determined: total soluble solids content, pH, titratable total acidity, color, chlorophyll content, protein, ash, lipids, and moisture. For the determination of total soluble solids, pH, and titratable total acidity, the samples were processed using a mixer–food processor model RI1341 (Philips Walita, China).

### Chlorophyll

For the determination of total chlorophyll, 1.0 g of leaf tissue was macerated in a mortar with 10 mL of acetone (80% concentration) to promote tissue disintegration, following the recommendation of Bruinsma (1963). After homogenization, 80% acetone was added to the extract until complete leaf discoloration was achieved, followed by filtration. The final volume of the extract was

adjusted to 50 mL. Absorbance readings were taken at 652 nm within 30 minutes after the start of extraction, with the extracts wrapped in aluminum foil to prevent light exposure. Total chlorophyll concentrations were determined in  $\text{mg}/100 \text{ g}$  of leaf tissue, according to the equation proposed by Engel and Poggiani (1991).

### Pigments contents

The contents of chlorophyll *a*, chlorophyll *b*, and carotenoids were determined according to the method proposed by Lichtenthaler (1987). For this purpose, 0.025 to 0.030 g of leaf discs from intermediate leaves were collected and transferred to Eppendorf tubes containing 1.5 mL of 80% acetone, in the absence of light. After 48 hours, absorbance readings were performed using a spectrophotometer at 663 nm (chlorophyll *a*), 647 nm (chlorophyll *b*), and 470 nm (carotenoids) (Figure 4).

Figure 4 – Stages of the laboratory analysis of pigment contents in lettuce samples.



Source: Prepared by the authors (2025).

The pigment contents were expressed in  $\mu\text{g/g}$  (Equations 1, 2, 3, and 4) (Figure 5). All procedures were carried out in a closed environment, avoiding direct exposure to light.

(1) Chlorophyll a =  $\text{Ca} = 12.25 \text{ A663} - 2.79 \text{ A647}$

(2) Chlorophyll b =  $\text{Cb} = 21.50 \text{ A647} - 5.10 \text{ A663}$

(3) Total chlorophylls =  $\text{C} (\text{a} + \text{b}) = 7.15 \text{ A663} + 18.71 \text{ A647}$

(4) Carotenoids (xanthophylls + carotenes) =  $(1000 \text{ A470} - 1.82 \text{ Ca} - 85.02 \text{ Cb}) / 198$

### Color

For the measurement of lettuce color, a Nix Color Sensor Pro colorimeter (NPRO; Nix Sensor Ltd., Burlington, Ontario, Canada) was used at three different points on the samples, employing the CIELab color system, which defines the chromatic space in rectangular coordinates ( $L^*$ ,  $a^*$ ,  $b^*$ ), where: (i)  $L^*$  represents lightness, ranging from 100 for perfectly white surfaces to 0 for black; (ii)  $a^*$  indicates the intensity of red (+) and green (-); and (iii)  $b^*$  indicates the intensity of yellow (+) and blue (-) (Figure 5).

### Characterization of Fish and Lettuce

Figure 5- Evaluation of leaf and fish coloration.

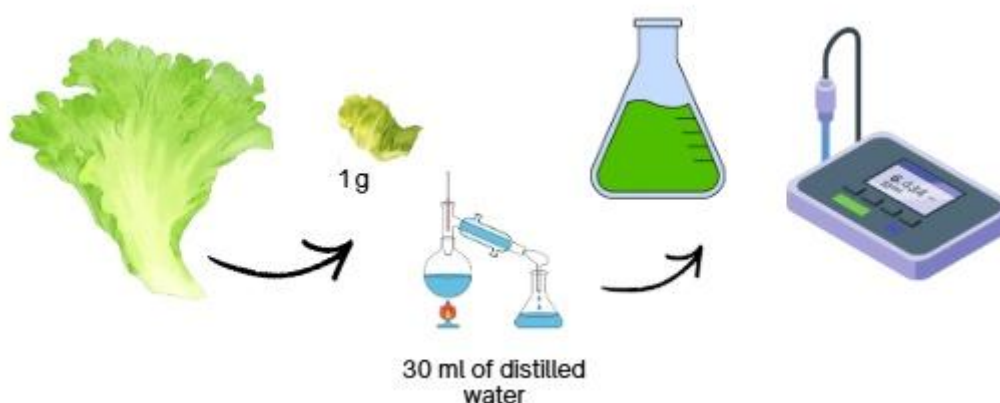


Source: Prepared by the authors (2025).

### pH

The pH of lettuce was determined by macerating 1.0 g of leaf tissue diluted in 30 mL of distilled water, using a digital potentiometer equipped with a glass membrane, in accordance with the methodology established by the Instituto Adolfo Lutz (2005). The instrument was calibrated with buffer solutions at pH 4.0 and 7.0 (Figure 6).

Figure 6- Evaluation of lettuce pH.



Source: Prepared by the authors (2025).

### Chemical Composition

For the chemical composition of fillets and lettuce, analyses of moisture (method no. 967.08), lipids (method no. 2003.06), ash (method no. 942.05), and protein (method no. 988.05) were performed according to the methodologies described by the Association of Official Analytical Chemists (AOAC, 2012). Moisture and ash contents were determined by gravimetric methods, namely

oven drying at  $105\text{ }^{\circ}\text{C}$  and incineration of the previously carbonized sample in a muffle furnace at  $550\text{ }^{\circ}\text{C}$ , respectively. Lipid content was determined according to Folch, Lees, and Stanley (1957). Protein content was estimated using the Kjeldahl method, applying a nitrogen conversion factor of 6.25. Fifteen random replicates of lettuce and fish samples were analyzed.

### Microbiological Analyses

Initially, 500 g of leaves from at least five lettuce heads were placed in a sterile plastic container. Subsequently, 25 g of this composite sample were randomly selected and subjected to microbiological analyses.

### Heterotrophic Bacterial Count

The heterotrophic bacterial count was performed using the spread-plate technique, in which 1 mL of the previously diluted and homogenized sample (for 60 s) was plated onto sterile plates containing Plate Count Agar (PCA). The plates were then incubated at  $36 \pm 1$  °C for 48 h. At the end of the incubation period, the presence or absence of microbial growth was observed, followed by colony counting using a colony counter. The number of colonies per plate after incubation was divided by the volume of sample transferred to the plate, and the results were expressed as the number of bacterial colonies per milliliter or colony-forming units (CFU/mL).

### Presumptive Test (Total Coliforms)

The presumptive test aims to identify microorganisms belonging to the coliform group, which, when inoculated into Lauryl Tryptose Broth, produce gas, indicating a positive presumptive result. The procedure involved inoculating the sample into a series of three tubes containing 10 mL, 9 mL, and 9.9 mL of Lauryl Tryptose Broth with the addition of 10 mL, 1 mL, and 0.1 mL of the collected sample, respectively. It is important to note that a Durham tube was placed in each tube. The tubes were then incubated in a bacteriological incubator at 37 °C for 48 h. Upon observation, the presence of gas in the Durham tube indicated a positive presumptive test, and the number of positive tubes for each dilution was recorded.

### RESULTS AND DISCUSSION

The values of chlorophyll, carotenoids, color, and pH are presented in Table 1. Color is an important marketing attribute and is recognized as a factor that attracts consumers; therefore, it is important to characterize the coloration of different cultivars, as well as to understand the physiological factors and processes that modify it (Batista *et al.*, 2015).

Table 1. Mean values and standard deviations of chlorophyll, carotenoids, color and pH.

| Variables                     | Means $\pm$ standard deviations |
|-------------------------------|---------------------------------|
| Total chlorophylls (mg/100 g) | 15.303 $\pm$ 2.185              |
| Chlorophyll a (mg/100 g)      | 13.488 $\pm$ 1.793              |
| Chlorophyll b (mg/100 g)      | 1.814 $\pm$ 0.393               |
| Carotenoids (mg/100 g)        | 607.021 $\pm$ 153.334           |
| L*                            | 41.609 $\pm$ 3.241              |
| a*                            | -12.011 $\pm$ 0.942             |
| b*                            | 24.697 $\pm$ 2.221              |
| pH                            | 6.696 $\pm$ 0.119               |

Pigmentation changes are highly relevant to lettuce quality. Chlorophyll loss in leafy vegetables represents a factor of major importance in the quality of minimally processed products during the period they are exposed on retail shelves (Silva *et al.*, 2007). Chlorophylls are hydrophobic structures whose primary function is to convert light energy into chemical energy, a process that occurs in chloroplasts (Streit *et al.*, 2005).

In the present study, total chlorophyll (15.303  $\pm$  2.185 mg/100 g), chlorophyll a (13.488  $\pm$  1.793 mg/100 g), chlorophyll b (1.814  $\pm$  0.393 mg/100 g), and carotenoids (607.021  $\pm$  153.334 mg/100 g) were observed. Lei and Engeseth (2021) reported higher levels of chlorophyll a and b in lettuce grown both in soil and hydroponically, with values of 24.81 mg/100 g and 20.53 mg/100 g for chlorophyll a, and 7.92 mg/100 g and 6.44 mg/100 g for chlorophyll b, respectively. Similar results were described

by Siomos *et al.* (2001), who also observed lower concentrations of chlorophyll a, b, and total chlorophyll in hydroponically grown lettuce compared with soil-grown lettuce. Conversely, Bohn *et al.* (2004) found values close to those of the present study when evaluating hydroponic lettuce grown in a floating system, reporting approximately 18 mg/100 g of total chlorophyll. Differences among studies may be associated with the genetic variability of cultivars, as highlighted by Cassetari *et al.* (2015), who stated that genetics directly influences chlorophyll content in lettuce. Similarly, Caldwell and Britz (2006) also reported cultivar-specific variations in chlorophyll content in greenhouse-grown green lettuce.

The L\*, a\*, and b\* values were 41.609  $\pm$  3.241, -12.011  $\pm$  0.942, and 24.697  $\pm$  2.221, respectively. Divergent results were observed for the Brunela cultivar evaluated by Fontana *et al.* (2018), who reported the

following color values:  $L^* = 67.7$ ,  $a^* = -17.3$ , and  $b^* = 36.1$ . Lower  $L^*$  values indicate darker samples. In a study conducted by Fontana *et al.* (2018) comparing conventional lettuce cv. Brunela with lettuce produced in hydroponic and organic systems, a statistically significant difference was observed, with organic samples showing lower  $L^*$  values (60.6, darker samples) and hydroponic samples showing higher  $L^*$  values (67.7, lighter samples). Overall, the results obtained in the present study demonstrate that lettuce cultivation in an MBT system does not negatively affect the evaluated luminosity parameters.

Higher  $a^*$  and  $b^*$  values are related to a greater presence of carotenoids, especially  $\beta$ -carotene, which has a yellow coloration (Faraoni *et al.*, 2009). According to Rocha and Reed (2014), color is determined by the presence of pigments, some of which may play an important role in health. The  $a^*$  and  $b^*$  values observed in this study are lower than those reported by Fontana *et al.* (2018). An increase in  $a^*$  intensity in lettuce represents a slight tendency toward red coloration. Higher  $b^*$  values indicate a greater presence of yellow components, and higher  $b^*$  values may be associated with the presence of carotenoids in lettuce, as these pigments are characterized by yellow, orange, and red colors and are present in fruits and vegetables (Silva *et al.*, 2010).

pH is an intrinsic factor of foods and is determined by the hydrogen ion concentration of the solution. pH allows the assessment of food deterioration, such as microbial growth, as well as flavor and odor retention and enzymatic activity (Lucena *et al.*, 2011). The pH values observed in this study were  $6.696 \pm 0.119$ , which fall within the range considered optimal for plant tissues, between 5 and 7 (Menezes *et al.*, 2005). Similar values were found by Covre *et al.* (2020), who reported a pH of 5.8.

Comparable values were reported by Martins *et al.* (2017), who observed pH values between 6.26 and 5.70 for the Cristal and Rubra cultivars, respectively, under hydroponic cultivation. Consistently, Sanches *et al.* (2017) recorded values between 5.37 and 5.40 for the Alcione, Amanda, and Caipira varieties. As highlighted by Freire *et al.* (2009), these pH variations are directly related to climatic conditions, plant nutritional status, and management practices, reinforcing that proper control of these factors is essential for the stability and performance of cultivation systems.

Proximate composition refers to the percentage quantification of the components of a given product, including water, protein, carbohydrates, lipids, fiber, and mineral residue or ash. The proximate composition values of lettuce and fish are presented in Table 2. The proximate composition of lettuce cultivated in aquaponic systems has been the subject of several studies.

According to Sgarbieri (1987), fresh soil-grown lettuce has the following average composition per 100 g of edible portion: water: 94%; protein: 1.3 g; fat: 0.3 g; total carbohydrates: 3.5 g; fiber: 0.7 g; calcium: 68 mg; phosphorus: 27 mg; iron: 1.4 mg; potassium: 264 mg; vitamin A: 1900 IU; thiamine: 0.05 mg; riboflavin: 0.08 mg; niacin: 0.4 mg; and vitamin C: 18 mg. Thus, the importance of lettuce as a good source of vitamins and mineral salts for human nutrition is unquestionable. Regarding moisture content, the values are comparable to those found in hydroponic systems, generally exceeding 94% (Almeida *et al.*, 2018). Oshe *et al.* (2001), evaluating the proximate composition of six hydroponic lettuce cultivars (Aurora, Brisa, Livia, Mimosa, Regina, and Verônica), did not observe differences in water content of the aerial parts, reporting an average value of 94.46%.

Table 2. Mean values and standard deviations of the proximate composition of lettuce and fish.

| Variables | Lettuce            | Fish               |
|-----------|--------------------|--------------------|
| Moisture  | $94.133 \pm 0.439$ | $78.810 \pm 0.822$ |
| Protein   | $2.200 \pm 0.482$  | $12.614 \pm 1.143$ |
| Lipids    | $2.492 \pm 0.428$  | $3.785 \pm 0.454$  |
| Ash       | $1.175 \pm 0.363$  | $1.558 \pm 0.265$  |

Regarding protein content, some studies suggest that aquaponic lettuce may exhibit slightly higher levels compared with hydroponic lettuce, possibly due to the availability of nitrogen derived from fish waste (Martins *et al.*, 2021). Oshe *et al.* (2009), evaluating the hydroponic cultivars Lucy Brown, Mimosa Verde, and Mimosa Vermelha, reported protein contents of  $0.83 \text{ g} \cdot 100 \text{ g}^{-1}$ ,  $1.28 \text{ g} \cdot 100 \text{ g}^{-1}$ , and  $1.15 \text{ g} \cdot 100 \text{ g}^{-1}$ , respectively. Matias *et al.* (2000), assessing estimated crude protein contents in different parts of organic, hydroponic, and conventional

lettuce plants, observed that protein levels generally ranged from 0.9 to 1.8% in organic lettuce, 1.7 to 3.1% in hydroponic lettuce, and 1.1 to 2.6% in conventional lettuce.

Lipid contents in aquaponic lettuce are also low, similarly to hydroponic and conventional cultivation systems (Costa *et al.*, 2019). However, in the present study, higher values were observed, which can be attributed to the methodology used for lipid quantification. This method also quantified non-lipid apolar substances,

such as carotenoids, cuticular waxes, hydrophobic pigments, some lipophilic phenols, membrane-bound phospholipid residues, and insoluble cellular fragments. Oshe *et al.* (2009), evaluating the hydroponic cultivars Lucy Brown, Mimosa Verde, and Mimosa Vermelha, reported lipid contents of 0.18, 0.42, and 0.42 g·100 g<sup>-1</sup>, respectively.

The ash content in aquaponic lettuce is likewise consistent with values observed in other soilless cultivation systems, indicating that aquaponics can produce lettuce with comparable nutritional value (Sousa & Oliveira, 2022).

Fish flesh has as its main chemical components water (50–85%), protein (12–24%), and lipids (0.1–22%), with approximately 2% distributed among minerals (0.08–2%), carbohydrates (0.1–3%), and vitamins (Britto *et al.*, 2014; Yarnpakdee *et al.*, 2014). These proportions vary according to species, age, sex, and nutrition. The proximate composition values of fish observed in this study fall within the expected range for the species: moisture (78.81 ± 0.822), protein (12.614 ± 1.143), lipids (3.785 ± 0.454), and ash (1.558 ± 0.265). Moisture and ash values are similar to those reported by Santos (2015), who evaluated tilapia fillets obtained from fish reared in earthen ponds and net cages, with moisture values of 79.39 ± 1.46 and 79.04 ± 1.41, and ash contents of 1.34 ± 0.38 and 1.55 ± 0.29, respectively. However, those authors reported higher protein values (19.42 ± 1.13 and 19.45 ± 1.27) and lower lipid contents (2.03 ± 0.98 and 2.00 ± 0.99) than those observed in the present study. Nevertheless, no significant differences were found between the proximate composition of tilapia reared in the two production systems. Overall, the proximate composition variables obtained in this study did not differ from those reported in the literature for the same species, even when reared in different systems. Albuquerque *et al.* (2004) reported moisture, protein, fat, and ash values of 81.05, 16.52, 1.98, and 1.14%, respectively, for tilapia fillets slaughtered on ice from a commercial farm in Ceará, Brazil. For tilapia from recreational fishing ponds in São Paulo, Simões *et al.* (2007) found values of 77.13, 19.36, 2.60, and 1.09%. Leonhardt *et al.* (2006) reported values of 76.88, 18.48, 2.96, and 1.41%, respectively, for Thai tilapia reared in net cages.

In integrated aquaculture systems combined with lettuce cultivation, the plant plays an essential role in filtering and removing dissolved nutrients from the water, particularly those derived from nitrogen excretion and phosphorus released by fish. This uptake contributes to improving water quality without generating competition or causing any negative impact on animal development. On the contrary, the use of lettuce in integrated systems tends to promote environmental balance and production efficiency, constituting a sustainable strategy that

complements aquaculture without compromising fish welfare or zootechnical performance.

According to Brazilian legislation, Normative Instruction No. 60 of December 23, 2019 (Brazil, 2019), regulated by ANVISA and establishing microbiological standards for foods, the maximum allowable level of thermotolerant coliforms is 10<sup>2</sup> g<sup>-1</sup> for fruits and vegetables. However, this regulation does not address heterotrophic bacterial counts, although it is known that high bacterial loads may pose risks to consumers (ANVISA, 2019).

Greenhouse cultivation, including hydroponically grown products, is considered safer with regard to microbiological issues when compared with other agricultural practices, due to the reduction of risk factors, including soil contact (Holvoet *et al.*, 2015; Sirsat & Neal, 2013). Nevertheless, hydroponic systems may be contaminated by pathogens originating from water, substrates, and human activity (Moriarty *et al.*, 2019; Shaw *et al.*, 2016). In the present study, no growth of any of the analyzed microorganisms was observed. Barnhart *et al.* (2015) evaluated microbial levels in leafy vegetables produced in aquaponic, hydroponic, and soil-based systems commercialized in the state of Minnesota (Minneapolis–Saint Paul metropolitan area and the Duluth region). Mohammad *et al.* (2022), when evaluating mean *E. coli* levels in organic lettuce samples, found no statistically significant differences among cultivation methods.

## Conclusion

Although still not widely adopted on a commercial scale in Brazil, aquaponics represents an excellent option for sustainable food production. Fish reared in aquaponic systems do not differ from those produced in other farming systems. Furthermore, analyses of vegetables produced in this system did not reveal any type of contaminant that would preclude their commercialization or consumption.

## References

- Agência Nacional de Vigilância Sanitária – ANVISA. (2019) Instrução Normativa nº 60, de 23 de dezembro de 2019. Estabelece as listas de padrões microbiológicos para alimentos. *Diário Oficial da União*, 26 dez. 2019, Seção 1, 133.
- Albuquerque, W.F.; Zapata, J.F.F.; Almeida, R.S. (2004) Estado de frescor, textura e composição muscular da tilápia do Nilo (*Oreochromis niloticus*) abatida com dióxido de carbono e armazenada em gelo. *Revista Ciência Agronômica*, 35, 264–271.
- Alves, T.N.; Echer, M.M.; Coutinho, P.W.R.; Menezes Júnior, E.K.; Klosowski, É.S.; Sackser, G.A.B.; Black,

- A.V. y Inagaki, A.M. (2020) Efeitos dos consórcios com quiabeiro nas características físico-químicas da couve de folha sob manejo orgânico. *Brazilian Journal of Development*, 6, 79987–80001.
- AOAC. (2012) *Official methods of analysis of AOAC International*. 19th ed. Gaithersburg: AOAC International.
- Azad, K.N.; Salam, M.A. y Azad, K.N. (2016) Aquaponics in Bangladesh: current status and future prospects. *Journal of Bioscience and Agriculture Research*, 7, 669–677.
- Bacha, H.; Tekaya, M.; Drine, S.; Guasmi, F.; Touil, L.; Enneb, H.; Triki, T.; Cheour, F. y Ferchichi, A. (2017) Impact of salt stress on morphophysiological and biochemical parameters of *Solanum lycopersicum* cv. Microtom leaves. *South African Journal of Botany*, 108, 364–369.
- Bailey, D.S. y Ferrarezi, R.S. (2017) Valuation of vegetable crops produced in the UVI commercial aquaponic system. *Aquaculture Reports*, 7, 77–82.
- Banerjee, G. y Ray, A.K. (2017) Bacterial symbiosis in the fish gut and its role in health and metabolism. *Symbiosis*, 72 (1), 1–11.
- Barnhart, C.; Hayes, L. y Ringle, D. (2015) Food safety hazards associated with smooth textured leafy greens produced in aquaponic, hydroponic, and soil-based systems. *University of Minnesota Aquaponics*, Minnesota.
- Batista, P.F.; Lima, M.A.C.; Trindade, D.C.G. y Alves, R.E. (2015) Quality of different tropical fruit cultivars produced in the Lower Basin of the São Francisco Valley. *Revista Ciência Agronômica*, 46 (1), 176–184.
- Beecher, L. (2021) *Aquaponics: system layout and components*. Clemson: Clemson Cooperative Extension.
- Bereded, N.K. et al. (2022) The gut bacterial microbiome of Nile tilapia (*Oreochromis niloticus*) from lakes across an altitudinal gradient. *BMC Microbiology*, 22 (1), 87.
- Bohn, T.; Walczyk, T.; Leisibach, S. y Hurrell, R.F. (2004) Chlorophyll-bound magnesium in commonly consumed vegetables and fruits: relevance to magnesium nutrition. *Journal of Food Science*, 69, 347–350.
- Britto, A.C.P.; Rocha, C.B.; Tavares, R.A.; Fernandes, J.M.; Piedras, S.R.N. y Pouey, J.L.O.F. (2014) Rendimento corporal e composição química do filé da viola (*Loricariichthys anus*). *Ciência Animal Brasileira*, 15 (1), 38–44.
- Caldwell, C.R. y Britz, S.J. (2006) Effect of supplemental ultraviolet radiation on the carotenoid and chlorophyll composition of greenhouse-grown leaf lettuce (*Lactuca sativa* L.). *Journal of Food Composition and Analysis*, 19 (6–7), 637–644.
- Cardinale, M. et al. (2015) Bacterial networks and co-occurrence relationships in the lettuce root microbiota. *Environmental Microbiology*, 17 (1), 239–252.
- Cassetari, L.S.; Gomes, M.S.; Santos, D.C.; Santiago, W.D.; Rade, J.; Guimarães, A.C. y Gomes, L.A. (2015) Carotene and chlorophyll levels in cultivars and breeding lines of lettuce. *Acta Horticulturae*, 1083, 469–473.
- Covre, E.A. et al. (2020) Caracterização físico-química e sensorial da alface Brunela. *Agrarian*, 13 (48), 265–272.
- Engel, V.L. y Poggiani, F. (1991) Estudo da concentração de clorofila nas folhas e seu espectro de absorção de luz em função de sombreamento em mudas de quatro espécies florestais. *Revista Brasileira de Fisiologia Vegetal*, 3 (1), 39–45.
- FAO. (2022) *The state of world fisheries and aquaculture*. Rome: FAO.
- Faraoni, A.S.; Ramos, A.M. y Stringheta, P.C. (2009) Caracterização da manga orgânica cultivar Ubá. *Revista Brasileira de Produtos Agroindustriais*, 11 (1), 9–14.
- Folch, J.; Lees, M. y Sloane Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226 (1), 497–509.
- Fontana, L. et al. (2018) Physicochemical characterization and sensory evaluation of lettuce cultivated in three growing systems. *Horticultura Brasileira*, 36 (1), 20–26.
- Freire, A.G.; Olivier, F.A.; Carrilho, M.J.S.O.; Oliveira, M.K.T. y Freitas, D.C. (2009) Qualidade de cultivares de alface produzidas em condições salinas. *Revista Caatinga*, 22 (4), 81–88.
- Hamom, A. et al. (2020) Identification of pathogenic bacteria from diseased Nile tilapia (*Oreochromis niloticus*) with their sensitivity to antibiotics. *International Journal of Current Microbiology and Applied Sciences*, 9 (3), 716–738.
- Holvoet, K. et al. (2015) Agricultural and management practices and bacterial contamination in greenhouse versus open field lettuce production. *International Journal of Environmental Research and Public Health*, 12 (1), 32–63.
- Instituto Adolfo Lutz. (1985) *Normas analíticas: métodos químicos e físicos para análise de alimentos*. 3 ed. São Paulo: IAL.
- Kasozi, N.; Kaiser, H. y Wilhelmi, B. (2022) Determination of phylloplane-associated bacteria of lettuce from a small-scale aquaponic system via 16S rRNA gene amplicon sequence analysis. *Horticulturae*, 8 (2), 151.
- Krastanova, M. et al. (2022) Aquaponic systems: biological and technological parameters.

- Biotechnology & Biotechnological Equipment*, 36 (1), 305–316.
- Lei, C. y Engeseth, N.J. (2021) Comparison of growth characteristics, functional qualities, and texture of hydroponically grown and soil-grown lettuce. *LWT – Food Science and Technology*, 150, 111931.
- Leonhardt, J.H.; Filho, M.C.; Forssard, H. y Moreno, A.M. (2006) Características morfológicas, rendimento e composição do filé de tilápia do Nilo (*Oreochromis niloticus*). *Semina: Ciências Agrárias*, 27 (1), 125–132.
- Lichtenthaler, H.K. (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology*, 148, 350–382.
- Lucena, E.M.P.; Assis, J.S.; Alves, R.E. y Filho, J.E. (2011) Alterações físico-químicas durante o crescimento e desenvolvimento de manga “Tommy Atkins” produzidas no vale do São Francisco, Brasil. *Journal of the Interamerican Society for Tropical Horticulture*, 53, 48–51.
- Magalhães, T.F.O. et al. (2022) Aquaponia: uma alternativa de agricultura de hortaliças com base na agroecologia. *Ciência Atual – Revista Científica Multidisciplinar do Centro Universitário São José*, 18.
- Martins, L.M.; Silva, E.C.; Carlos, L.A.; Ferraz, L.C.L.; Maciel, G.M. y Cruz, J.L. (2017) Physical and chemical characteristics of lettuce cultivars grown under three production systems. *Bioscience Journal*, 33 (3), 621–630.
- Martins, T.S. (2021) Desempenho agrônomo de cultivares de alface em sistema hidropônico no município de Uruçui-PI. Uruçui: IFPB.
- Menezes, E.M.S.; Fernandes, E.C. y Sabaa-Srur, A.U.O. (2005) Folhas de alface lisa (*Lactuca sativa*) minimamente processadas armazenadas em atmosfera modificada: análises físicas, químicas e físico-químicas. *Revista Ciência e Tecnologia de Alimentos*, 25 (1), 60–62.
- Mohammad, Z.H.; Prado, I. do y Sirsat, S.A. (2022) Comparative microbial analyses of hydroponic versus in-soil grown Romaine lettuce obtained at retail. *Heliyon*, 8 (10).
- Monsees, H. et al. (2019) Lettuce (*Lactuca sativa*, variety Salanova) production in decoupled aquaponic systems: same yield and similar quality as in conventional hydroponic systems but drastically reduced greenhouse gas emissions by saving inorganic fertilizer. *PLOS One*, 14 (6), e0218368.
- Moriarty, M.J. et al. (2019) Internalization assessment of *Escherichia coli* O157:H7 in hydroponically grown lettuce. *LWT – Food Science and Technology*, 100, 183–188.
- Ohse, S. et al. (2001) Qualidade de cultivares de alface produzidos em hidroponia. *Scientia Agrícola*, 58, 181–185.
- Rocha, D.S. y Reed, E. (2014) Pigmentos naturais em alimentos e sua importância para a saúde. *Estudos Vida e Saúde*, 76–85.
- Ross, L.G. y Ross, B. (2008) *Anaesthetic and sedative techniques for aquatic animals*. Oxford: Blackwell Publishing.
- Sanches, A.G.; Silva, M.B.; Moreira, E.G.S.; Costa, J.M.; Cosme, S.S. y Cordeiro, C.A.M. (2017) Avaliação da qualidade de alfaces minimamente processadas cultivadas em sistema hidropônico. *Revista Trópica – Ciências Agrárias e Biológicas*, 9 (1), 19–31.
- Santos, V.G.N. et al. (2015) Rendimento corporal, composição centesimal e resistência do couro de tilápia (*Oreochromis niloticus*) produzida em viveiros escavados e tanques-rede.
- Sawyer, T. (2021) Food safety and *Escherichia coli* in aquaponic and hydroponic systems. *Horticulturae*, 7 (3), 36.
- Sgarbieri, V.C. (1987) *Alimentação e nutrição: fator de saúde e desenvolvimento*. Campinas: UNICAMP.
- Shaw, A. et al. (2016) Growth of *Escherichia coli* O157:H7, non-O157 Shiga toxin-producing *Escherichia coli*, and *Salmonella* in water and hydroponic fertilizer solutions. *Journal of Food Protection*, 79 (12), 2179–2183.
- Silva, J.M.; Ongarelli, M.G.; Aguila, J.S.D.; Sasaki, F.F. y Kluge, R.A. (2007) Métodos de determinação de clorofila em alface e cebolinha minimamente processadas. *Revista Iberoamericana de Tecnología Postcosecha*, 8 (2), 53–59.
- Simões, M.R.; Ribeiro, C.F.A.; Ribeiro, S.C.A.; Park, K.J. y Murr, F.E.X. (2007) Composição físico-química, microbiológica e rendimento do filé de tilápia tailandesa (*Oreochromis niloticus*). *Ciência e Tecnologia de Alimentos*, 27 (3), 608–613.
- Siomos, A.S.; Beis, G.; Papadopoulou, P.P. y Barbayiannis, N. (2001) Quality and composition of lettuce (cv. ‘Plenty’) grown in soil and soilless culture. *Acta Horticulturae*, 548 (52), 445–450.
- Sirakov, I. et al. (2016) Potential for combined biocontrol activity against fungal fish and plant pathogens by bacterial isolates from a model aquaponic system. *Water*, 8 (11), 518.
- Sirsat, S.A. y Neal, J.A. (2013) Microbial profile of soil-free versus in-soil grown lettuce and intervention methodologies to combat pathogen surrogates and spoilage microorganisms on lettuce. *Foods*, 2 (4), 488–498.
- Streit, N.M.; Canterle, L.P.; Canto, M.W. y Hecktheuer, L.H.H. (2005) As clorofilas. *Ciência Rural*, 35 (3), 748–755.

- Sutani, J. et al. (2021) Avaliação do desempenho produtivo de cultivares de alfaces em sistemas aquapônico e hidropônico. *Cadernos de Ciência & Tecnologia*, 38 (3), e26909.
- Ventura, C.; Guilherme, D.O.; Vieira, E.T.V. y Baumann, M.G. (2019) Aquaponia como alternativa de produção sustentável à agricultura familiar urbana. In: Andrade Junior, J.M. (org.) *Sustentabilidade, globalização e inovação*. São Paulo: Editora Inovar, 54–70.
- Verdoliva, S.G. et al. (2021) Controlled comparisons between soil and hydroponic systems reveal increased water use efficiency and higher lycopene and  $\beta$ -carotene contents in hydroponically grown tomatoes. *Scientia Horticulturae*, 279, 109896.
- Wielgosz, Z.J.; Anderson, T.S. y Timmons, M.B. (2017) Microbial effects on the production of aquaponically grown lettuce. *Horticulturae*, 3 (3), 46.
- Wirza, R. y Nazir, S. (2021) Urban aquaponics farming and cities: a systematic literature review. *Reviews on Environmental Health*, 36 (1), 47–61.
- Yarnpakdee, S.; Benjakul, S.; Penjamras, P. y Kristinsson, G.H. (2014) Chemical compositions and muddy flavour/odour of protein hydrolysate from Nile tilapia and broadhead catfish mince and protein isolate. *Food Chemistry*, 142, 210–216.