

Article

Comprehensive Characterization of the Nutritional Composition, Mineral Profile, Phytochemical Characteristics, and Antioxidant Capacity of Aquaponically Grown Red Amaranth (*Amaranthus cruentus* L.)

Neli Grozeva ^{1,2,*}, Galina Gospodinova ³, Roksana Mineva ³, Denitsa Georgieva ⁴, Silviya Hristova ⁴, Milena Tzanova ¹, Svetoslava Terzieva ¹, Georgi Beev ^{1,2}, Neven Terziev ⁵, Daniela Tsvetanova Stoeva ¹ and Zvezdelina Yaneva ⁴

¹ Department of Biological Sciences, Faculty of Agriculture, Trakia University, Students Campus, 6000 Stara Zagora, Bulgaria; milena.tsanova@trakia-uni.bg (M.T.); svetoslava.terzieva@trakia-uni.bg (S.T.); georgi.beev@trakia-uni.bg (G.B.); daniela.stoeva@trakia-uni.bg (D.T.S.)

² Center of Competence AgriFood Systems and Bioeconomy, 4000 Plovdiv, Bulgaria

³ Department of Plant Science, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria; galina.gospodinova@trakia-uni.bg (G.G.); roksana.mineva@trakia-uni.bg (R.M.)

⁴ Department of Pharmacology, Animal Physiology, Biochemistry and Chemistry, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria; denitsa.georgieva@trakia-uni.bg (D.G.); silviya.hristova@trakia-uni.bg (S.H.); zvezdelina.yaneva@trakia-uni.bg (Z.Y.)

⁵ Aqua Field Ltd., 6000 Stara Zagora, Bulgaria; neven.terziev.23@trakia-uni.bg

* Correspondence: n.grozeva@trakia-uni.bg

Abstract

Aquaponics is an integrated and resource-efficient production system that combines aquaculture and hydroponics in a closed-loop environment with reduced water consumption and nutrient losses. The present study evaluated the nutritional composition, mineral profile, microbiological quality, and antioxidant-related phytochemical characteristics of red amaranth (*Amaranthus cruentus* L.) cultivated in a recirculating aquaponic system under controlled environmental conditions. Leaf biomass was analyzed for proximate composition, macro- and micronutrient content, total phenolic and flavonoid compounds, betalains, chlorophyll pigments, and antioxidant activity using standard analytical and spectrophotometric methods. The results demonstrated high crude protein content and substantial accumulation of essential minerals, particularly calcium, potassium, and magnesium. The analyzed biomass also exhibited elevated levels of phenolic compounds, flavonoids, betalains, and chlorophyll pigments associated with considerable antioxidant potential. The pigment profile suggested good physiological adaptation of plants to aquaponic cultivation conditions. In addition, microbiological analysis confirmed acceptable hygienic quality and safety of the harvested plant material. Overall, the findings indicate that red amaranth can be successfully cultivated in aquaponic systems while maintaining high nutritional value and functional food potential. The study highlights aquaponic cultivation as a sustainable approach to producing nutrient-dense leafy vegetables within environmentally responsible agricultural systems.

Keywords: recirculating aquaculture system (RAS); *Amaranthus cruentus* L.; antioxidant activity; bioactive compounds; mineral composition; sustainable agriculture; functional food; resource-efficient production; circular bioeconomy

Received: 2 June 2026

Revised: 2 July 2026

Accepted: 5 July 2026

Published: 7 July 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons](#)

[Attribution \(CC BY\)](#) license.

1. Introduction

In the context of accelerating urbanization, ongoing degradation of soil resources, and the increasing global demand for food, humanity is increasingly compelled to systematically reconsider conventional approaches to agricultural production [1]. Conventional agriculture is characterized by high resource intensity and is responsible for approximately 70% of global freshwater withdrawals at the planetary scale [2]. This sector also represents a major contributor to surface water eutrophication due to diffuse and often uncontrolled runoff of nitrogen- and phosphorus-based compounds into aquatic ecosystems. Such environmental pressures are widely recognized as critical consequences of intensive agricultural production systems and their associated nutrient loading dynamics [3,4]. Within the European Union, these challenges are being addressed through strategic policy frameworks such as the European Green Deal and the Farm to Fork Strategy, which aim to reduce nutrient losses by at least 50% and fertilizer use by 20% by 2030 [5].

Against this regulatory and environmental background, aquaponics has emerged as an innovative approach to integrated food production. By combining recirculating aquaculture systems (RAS) with hydroponic cultivation of leafy vegetables and other crops, aquaponics embodies the principles of the circular economy while enhancing resource-use efficiency [6]. In addition to improving the efficiency of water and nutrient utilization, these systems reduce the environmental footprint of agricultural production by converting waste products from one biological process into valuable resources for another [7,8]. Recent studies further highlight aquaponics as a climate-resilient production system with potential to decouple food production from soil dependency and reduce external fertilizer inputs [6,7,9].

Among leafy vegetable crops, *Amaranthus* spp. (Amaranthaceae) exhibit rapid growth, high adaptability, and efficient nutrient uptake from recirculating water, characteristics that make them suitable for aquaponic cultivation systems [10,11]. Moreover, species within the genus *Amaranthus* L. are characterized by high levels of bioactive phytochemicals and antioxidant compounds, supporting their growing importance as functional foods with added nutritional and health-promoting value [12,13]. In particular, *A. cruentus* L., an annual herbaceous species native to Central America, has been identified as especially suitable for aquaponic cultivation. Its integration into aquaponic systems offers significant opportunities for sustainable production of leafy vegetables while simultaneously providing ecological, nutritional, and economic benefits. However, despite its agronomic potential, systematic data on how aquaponic cultivation modulates its biochemical composition remain scarce [7,9,14].

In particular, limited evidence exists regarding how controlled nutrient recycling in aquaponic systems influences secondary metabolite synthesis, antioxidant activity, and overall functional food quality in *A. cruentus* [7]. Addressing this gap is essential for understanding plant physiological responses under circular production conditions and for optimizing cultivation strategies aimed at enhancing nutritional value [6,14].

The aim of the present study is to investigate the potential of *A. cruentus* cultivated in a recirculating aquaponic system, with particular emphasis on evaluating the influence of aquaponic cultivation on its characteristics as a functional food, including its nutritional and bioactive compound profile.

By integrating agronomic performance with phytochemical and functional quality assessment, this study contributes to the broader understanding of how circular production systems can simultaneously enhance productivity, resource efficiency, and food quality. The findings are expected to support the development of evidence-based guidelines for the optimization of leafy vegetable production in aquaponic systems.

2. Materials and Methods

2.1. Experimental Design

The study was conducted at the Aquaponics Center established through a collaboration between “AKVA FIELD” Ltd. and Trakia University. The experimental period spanned from December 2025 to April 2026, and all data presented in this study were collected during this period. The facility incorporates an integrated aquaponic system for the combined production of freshwater fish and leafy vegetables, based on Recirculating Aquaculture Systems (RAS) and organic hydroponic cultivation under strictly controlled conditions. The production cycle is fully integrated and characterized by super-intensive cultivation technology.

The recirculating aquaculture system was designed for intensive production, maintaining constant water and aeration flow rates, stable water volume, and continuous operation of mechanical and biological filtration units. The total capacity of the aquaculture facility is 155.5 m² and 43 m³, distributed among five functional subsystems with varying areas and volumes. Water was continuously supplied in a closed loop, flowing from the fish tanks to the plant beds and pumped back for reuse. Before reaching the plants, the water underwent multi-stage filtration (clarifiers, mineralization, filters, and degassing), while no additional filter was installed at the plant outlet as the plants themselves served as the final biological purification.

In the present study, Nile tilapia (*Oreochromis niloticus*) were purchased from the Experimental Base of Trakia University, Stara Zagora, Bulgaria. Tilapia (*Oreochromis niloticus*) were cultivated under controlled environmental conditions, including a stable water temperature of approximately 25 °C, constant pH, and optimal dissolved oxygen levels. Water circulation was maintained at 6 m³/h for each of the two main recirculating systems, allowing complete water turnover approximately every hour. The water used in the system exhibited stable physicochemical characteristics and required only minimal replacement, thereby reducing the risk of external contamination. Water quality was regularly monitored throughout the experimental period by assessing the main physicochemical parameters as described in Section 2.2.

Dissolved oxygen concentration was maintained at approximately 8 mg/L, with intensified monitoring during feeding periods. The system comprised 12 tanks with a total working volume of 24 m³ and a maximum biomass density of 40 kg/m³ at an average fish weight of 350 g, providing a production capacity of up to 960 kg per production cycle. The application of RAS technology contributes to extended equipment lifespan, reduced antibiotic use, and the production of high-quality fish biomass.

Plants were cultivated under controlled greenhouse conditions. During the experimental period, average daytime temperatures of 20 ± 3 °C and nighttime temperatures of 16 ± 2 °C were maintained, while relative humidity ranged from 60 to 80%.

The plant component of the aquaponic system consisted of four large floating raft beds, six substrate-based beds for leafy vegetable cultivation, which also served as biological water purifiers, and seven smaller water beds. Seedling production and early plant development were performed using the Nutrient Film Technique (NFT), in which plants are cultivated in a thin layer of circulating nutrient solution, a widely established method in hydroponic and aquaponic production systems.

In the floating raft system, water is continuously recirculated from the fish tanks to shallow cultivation beds filled with water. The plants were positioned on floating rafts, allowing their root systems to remain in constant contact with water enriched with dissolved mineral nutrients in their plant-available ionic forms, originating entirely from organic sources via fish metabolic waste and commercial feed mineralization. Crucially,

no synthetic fertilizers or chemical nutrient supplements were utilized at any stage of the experiment to prepare or amend the nutrient solution for amaranth cultivation. Throughout the entire 4–5 month experimental period, no exogenous chemical nutrients were added to replenish the solution; instead, nutrient concentrations were dynamically and continuously maintained by the continuous biological loading of the aquaculture component. Fresh water was added periodically solely to compensate for evapotranspiration losses and to sustain a constant hydroponic operational level. The plant cultivation and filtration loop has a total nutrient solution reservoir capacity of 19 m³, establishing a specific allocation ratio of approximately 20 L of available nutrient solution per individual amaranth plant. The initial system water exhibited a baseline Electrical Conductivity (EC) of 0.4 mS/cm, which progressively increased and stabilized within an operational range of 1.2–1.6 mS/cm during active plant growth, reflecting a balanced nutrient profile available for amaranth uptake. All essential macro- and micronutrients required for plant growth were derived exclusively from the fish metabolic waste and the biological mineralization of the commercial fish feed within the recirculating system. The selected production model is recognized as an efficient and scalable approach, supported by extensive experimental data and scientific research.

Red amaranth (*A. cruentus*) is an annual herbaceous plant belonging to the family Amaranthaceae and cultivated as a leafy vegetable crop. The plant is characterized by an erect stem and simple leaves with an intense red to purple coloration resulting from the presence of anthocyanin pigments. In the present study, seeds supplied by Microgreens Bulgaria were used.

To clarify the cultivation methodology and address the distinction between substrate-based and substrate-less management, the plants were not divided into separate, parallel experimental treatments. Instead, all amaranth individuals progressed sequentially through different cultivation subsystems that corresponded to their specific chronological growth phases within a single connected system loop. Plant developmental stages were defined according to the BBCH scale [15]. Seeds were sown in rockwool cubes and placed in a shallow layer of recirculating nutrient solution during early plant development (BBCH 00–09) (Figure 1A). Plants were maintained under these conditions until reaching the leaf development stage, characterized by the development of 3–4 true leaves (BBCH 13–14) (Figure 1B). On the 58th day after sowing, plants were transferred to Nutrient Film Technique (NFT) channels for further cultivation (Figure 1C). On the 83rd day after sowing, plants were transferred to floating raft beds (Figure 1D). On the 153rd day after sowing, plants reached the stage of visible inflorescence (BBCH 51–52) (Figure 1E) and were relocated to substrate beds containing expanded clay media (Figure 1F).

2.2. Water Quality Parameters

Water samples were harvested from the system's circulation line under steady-state operating conditions using sterile polyethylene containers, which were pre-rinsed with the process water. Sampling occurred at two distinct phenological intervals: (i) seeding stage—the initial collection phase, and (ii) true leaf stage—collected at BBCH scales 11–12. The latter stage is defined by the transition from germination to active seedling growth, characterized by the presence of 1–2 true leaves morphologically similar to mature *A. cruentus*. To preserve chemical integrity and mitigate compositional shifts, all analyses were conducted immediately following collection. Quantitative determination of ammonium nitrogen (NH₄⁺-N), nitrite nitrogen (NO₂⁻-N), phosphate (PO₄³⁻-P), total water hardness (Ca²⁺/Mg²⁺), and aluminum concentration (Al³⁺) was performed via spectrophotometry. These measurements utilized a DR 5000 UV/Vis spectrophotometer (Hach-Lange, Düsseldorf, Germany) in conjunction with standardized Hach-Lange reagent kits, strictly adhering to the manufacturer's analytical protocols. Additionally,

permanganate oxidizability (in mg/dm³) was quantified through titrimetric analysis with potassium permanganate (KMnO₄).

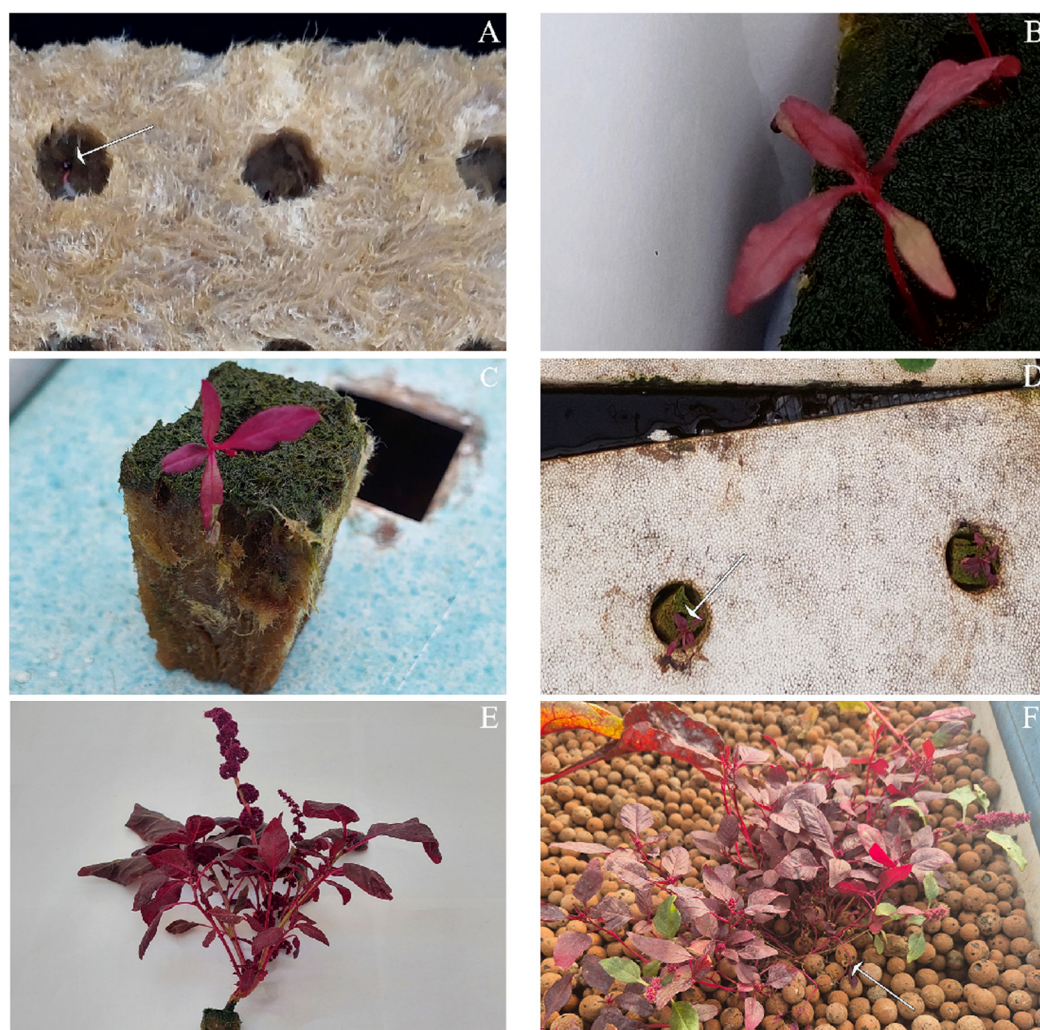


Figure 1. Developmental stages and cultivation phases of *A. cruentus* in the aquaponic system: (A) seeds sown in rockwool cubes; (B) plants at the leaf development stage (3–4 true leaves); (C) plants during transfer to NFT channels; (D) plants transferred to floating raft beds; (E) plants at the visible inflorescence stage; (F) cultivation in substrate beds containing expanded clay media.

The microbiological composition of the water from the two tanks of the recirculation system (S1 and S2) and the vegetable bed in the aquaponic system was performed by aseptic sampling in sterile containers “Nasko-WHIRL-PAK” (Whirl-Pak®, Pleasant Prairie, WI, USA). The samples were transported in a refrigerated bag and analyzed immediately in the microbiology laboratory. The following indicators were monitored: total number of mesophilic microorganisms, presence of *Escherichia coli*, total number of coliform bacteria, and presence of enterococci. For the indicator Total number of mesophilic microorganisms in 1 mL of the tested water, decimal serial dilutions were initially performed in pre-sterilized tubes with saline solution (NaCl 0.9% w/v). Subsequently, 1 mL of each dilution was directly inoculated onto ready-to-use Compact Dry TC plates with culture medium (R-Biopharm AG, Pfungstadt, Germany) in a laminar flow hood, incubated at 37 °C for 24–48 h, and the grown colonies were counted. All results were expressed as colony-forming units (CFU/mL).

A vacuum filtration system was used to determine *E. coli*, total coliforms, and enterococci. Volumes of 100 mL of the tested water were filtered through a sterile membrane filter with a size of 0.45 μm . The membrane filters were placed on specific Compact Dry ETC and Compact Dry EC plates, respectively, for enterococci, *E. coli*, and total coliforms. The plates were incubated for 24 h at 37 °C for the EC, and 24–48 h for ETC, and after that, colonies with a typical color were counted. The results obtained were converted to 1 mL of water (CFU/mL).

2.3. Plant Material

Samples for the present study were collected at full commercial maturity, defined as the stage of well-developed leaves prior to flowering. Leaf samples were thoroughly washed with running water, air-dried, and subsequently subjected to artificial drying at 40 °C. After completion of the drying process, the samples were prepared for further analyses, including quantitative determination of mineral and organic compounds, in order to provide comprehensive information regarding the potential of this plant as a raw material for the production of “superfoods”.

2.4. Methods for Plant Material Analysis

Chemical analyses of leaf samples from red amaranth (*A. cruentus*) were performed at the Research Laboratory Complex of the Faculty of Agriculture, Trakia University.

2.4.1. Determination of Moisture and Dry Matter

Moisture content was determined gravimetrically following the protocol of ISO 6496:2000 [16]. Dry matter content was calculated as the difference from 100%.

2.4.2. Determination of Total Nitrogen and Crude Protein

Total nitrogen content was determined using the Kjeldahl method according to ISO 5983-1:2006 [17]. The procedure includes mineralization with sulfuric acid, followed by ammonia distillation and titrimetric determination. Crude protein content was calculated based on the obtained nitrogen values.

2.4.3. Determination of Crude Fat

Crude fat content was determined according to the procedure described in ISO 6492:2007 [18]. The method involves Soxhlet extraction using an organic solvent and gravimetric determination of the dry extract.

2.4.4. Determination of Nitrogen-Free Extract (NFE)

Nitrogen-free extract (NFE) was calculated by difference and expressed as a percentage of dry matter.

2.4.5. Determination of Crude Fiber

Crude fiber content was determined using an in-house validated method (IVM 3/2024), which includes sequential acid and alkaline hydrolysis of a defined amount of plant material. The dry residue was determined gravimetrically.

2.4.6. Determination of Crude Ash

Crude ash content was determined in accordance with ISO 5984:2022 [19] by incineration of samples until complete removal of organic matter, with the resulting mineral residue determined gravimetrically.

2.4.7. Determination of Mineral Composition

Samples were digested using microwave-assisted closed-vessel digestion with nitric acid and hydrogen peroxide. The concentrations of Ca, Mg, K, P, Mn, Zn, Fe, and Cu were determined by ICP-AES (Inductively Coupled Plasma Optical Emission Spectroscopy) multi-element analysis following EPA 3051/3052 protocols. The instrument used was a PerkinElmer Optima 5300DV (PerkinElmer, Shelton, CT, USA).

2.4.8. Determination of Photosynthetic Pigments

Chlorophyll a, chlorophyll b, and total chlorophyll were extracted from plant tissues using 95% ethanol. Absorbance of the resulting extracts was recorded at 664 and 649 nm using a DR5000 UV-VIS spectrophotometer (Hach Lange, Düsseldorf, Germany). Concentrations were quantified according to the equations established by Lichtenthaler and Wellburn [20].

2.4.9. Secondary Metabolites Profile

Red amaranth leaves, dried to a constant weight, were subjected to solvent extraction in 70% ethanol at a 1:10 (*w/v*) ratio. The extraction was facilitated by ultrasonication for 30 min. Post-extraction, the homogenate was filtered through and stored in airtight containers at 4 °C until subsequent analysis.

- Total Phenolic Content (TPC)

The TPC was determined spectrophotometrically at 765 nm using a DR5000 UV-VIS spectrophotometer, following the Folin–Ciocalteu method as described by Yaneva et al. [21]. The experimental results are reported as mg of gallic acid equivalents per g of dry weight (mg GAE/g DW).

- Total Flavonoid Content (TFC)

The TFC was evaluated spectrophotometrically via the AlCl_3 colorimetric assay, based on a modified procedure by Dinev et al. [22]. TFC was expressed as mg of quercetin equivalents per g of dry weight (mg QE/g DW).

- Total Betalain Content (TBC)

The TBC in red amaranth extracts was quantified spectrophotometrically by measuring the concentrations of red-violet betacyanins (BC) and yellow-orange betaxanthins (BX). The absorbance of the appropriately diluted supernatant was recorded at 538 nm for betacyanins and 480 nm for betaxanthins using a DR 5000 UV-VIS spectrophotometer (Hach Lange, Germany). The pigment concentrations were calculated based on the Beer-Lambert law using the following equation [23]:

$$BC/BX = \frac{A_{538/480} \cdot D_F \cdot M_R \cdot V \cdot 1000}{\epsilon \cdot L \cdot W}, \text{ mg/g DW} \quad (1)$$

where *BC* is the betacyanins concentration; *BX*—the betaxanthins concentration; $A_{538/480}$ is the absorbance at the respective maximum wavelength for betacyanins and betaxanthins; D_F —the dilution factor; M_R —the molecular weight (550 g/mol for betanin and 308 g/mol for vulgaxanthin-I); *V*—the total extract volume (L); ϵ —the molar extinction coefficient (60,000 L/(mol·cm) for betanin and 48,000 L/(mol·cm) for vulgaxanthin-I); *L*—the path length of the cuvette (1 cm); *W*—the dry weight of the sample (g).

The total betalain content was expressed as the sum of BC and BX, with results reported as milligrams of pigment per gram of dry weight (mg/g DW).

2.4.10. Antioxidant Capacity Assays

- DPPH Radical Scavenging Activity

Briefly, 200 µL of red amaranth extract was reacted with a DPPH working solution and incubated in the dark at ambient temperature for 30 min. Absorbance was recorded at 517 nm [24]. The antioxidant activity was calculated as the percentage of DPPH inhibition [25].

- ABTS Radical Scavenging Assay

ABTS radical cation was generated by mixing ABTS and potassium persulfate, followed by dilution to an absorbance of 0.700 at 734 nm. Sample aliquot of the plant extract were added to the solution and incubated for 5–7 min. The obtained results were expressed as the percentage of ABTS inhibition according to Yaneva et al. [21].

- FRAP Assay

The Ferric Reducing Antioxidant Power (FRAP) assay measures the reduction in ferric-tripyridyltriazine (Fe^{3+} -TPTZ) to the ferrous form (Fe^{2+} -TPTZ) at low pH, which is associated with the formation of intensive blue color. The FRAP reagent was added to sample aliquots, subsequently incubated for 4 min at 37 °C, and the absorbance at 593 nm was measured. Results were calculated by comparing the absorbance change to a standard curve of FeSO_4 solution with known concentrations and expressed as mg FeSO_4 /g DW [26].

2.5. Statistical Analysis

All analytical measurements were performed in triplicate, and the obtained results are presented as mean values $\pm$ standard deviation (SD). Descriptive statistical analysis was applied for the evaluation of physicochemical, microbiological, mineral, and phytochemical parameters. Data processing and graphical presentation were performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

3. Results and Discussion

3.1. Water Quality Analyses

Based on the obtained water quality data presented in Table 1, the following section evaluates the system's performance for red amaranth (*A. cruentus*) cultivation, comparing these findings with literature benchmarks for soil and hydroponic systems. Particular emphasis is placed on understanding the continuous, biological replenishment of macronutrients and the systemic dynamics of Electrical Conductivity (EC) and pH as drivers of plant mineral assimilation.

Table 1. Values of the physicochemical parameters of aquaponic water during the sowing and true leaf stages of red amaranth cultivation.

Parameter	Sowing Stage	True Leaf Stage	Recommended Limit	Scientific Basis	Ref.
$\text{NH}_4\text{-N}$, mg/L	nd *	nd *	<1.0	High levels are toxic to fish and indicative of biofilter failure. At pH < 7.0, non-toxic NH_4^+ is prevailing; At pH > 7.0, it converts to toxic NH_3	[27,28]

NO ₂ -N, mg/L	0.130 ± 0.005	0.173 ± 0.006	<1.0	Nitrite inhibits oxygen transport in fish ("brown blood disease"); Levels should ideally be approximately zero in established systems.	[29]
NO ₂ ⁻ , mg/L	0.428 ± 0.009	0.572 ± 0.012			
PO ₄ ³⁻ -P, mg/L	nd *	nd *	30–100	While aquaponics often run at "undetectable" levels, commercial hydroponic benchmarks for leafy green plants suggest the cited range for maximum biomass production.	[30]
Permanganate oxidizability, mg O ₂ /L	3.84 ± 0.08	4.64 ± 0.1	<10.0	Levels > 10 mg/L in recirculating systems can lead to oxygen depletion and bacterial blooms.	[31]

* nd = non-detectable (below instrument detection limits).

3.1.1. Nitrogen Dynamics and Nitrification Efficiency

In a functional aquaponics system, fish produce ammonia, which bacteria convert into nitrite and finally into nitrate, which is the form usable by plants [27].

In a well-managed system using *Tilapia*, total ammonia nitrogen has to be maintained at low levels, with optimal conditions within the range 0.12 mg/L–0.32 mg/L [28]. Regarding nutrient uptake, red amaranth acts as an effective nutrient sink, keeping water quality within tolerance ranges for fish, with studies indicating it excels at converting nitrogen from fish waste into edible biomass [10].

The analysis of the results in the present study revealed that ammonium nitrogen (NH₄-N and NH₄⁺) was below detectable limits ("nd") during both the sowing and true leaf stages. This absence of measurable ammonia indicates a highly efficient biofiltration process, where nitrifying bacteria rapidly convert toxic ammonia excreted by fish into less harmful nitrogenous forms [32,33]. This complete conversion is further supported by the stable pH values (7.22 to 7.15), which optimized the physiological performance of nitrifying autotrophs without inducing ammonia toxicity.

Conversely, nitrite nitrogen (NO₂-N) was detected at low but measurable levels of 0.173 ± 0.006 mg/L and 0.130 ± 0.005 mg/L in the true leaf and sowing stages, respectively. While these values increased slightly as the plants matured (true leaf stage), they remain well below the toxic threshold for most aquaponic crops and fish species. In typical aquaponic systems, nitrite levels should ideally be maintained below 1.0 mg/L to prevent "brown blood disease" in fish and root inhibition in plants [34]. The slight increase during the true leaf stage may correspond to increased feeding rates to support the growing fish biomass, which in turn increases the nitrogen load on the system [30,35].

3.1.2. Phosphorus and Nutrient Availability

The measured phosphate (PO₄³⁻-P) levels in the present study were consistently below detectable limits during both growth stages. This is a common observation in aquaponic systems compared to hydroponics, where phosphate is often maintained at concentrations between 30 and 100 mg/L via the application of chemical fertilizers. It is critical to note that the "non-detectable" status of free orthophosphate in the bulk water does not imply a nutritional deficiency. Dissolved nutrients derived from organic sources (fish feed and feces) are present in highly bioavailable ionic forms. In aquaponics, plants like red amaranth must rely on the mineralization of fish waste and feed to obtain phosphorus. Because red amaranth has high nutritional demands, often requiring up to 90–150 kg N/ha in soil-based systems for optimal yield, the undetectable phosphate in the water suggests a highly dynamic equilibrium. Plants sequester available orthophosphates instantly upon their generation through biological mineralization, leaving negligible trace

concentrations in the water column while accumulating substantial phosphorus in their tissues [36]. This robust accumulation and active nutrient availability are further validated by the system's Electrical Conductivity (EC). Starting from a baseline system water value, the EC increased from 0.42 ± 0.02 mS/cm during the initial sowing phase to a highly productive operational range of 1.35 ± 0.05 mS/cm during the true leaf stage. This progressive increase reflects the steady accumulation of dissolved mineral ions generated by fish metabolic processes and feed degradation, satisfying the nutritional requirements of *A. cruentus* without the addition of any synthetic inputs. In addition, as a plant with high nutritional demand, red amaranth efficiently absorbs orthophosphates and NO_3^- -N from fish waste, helping to sustain water quality within optimal ranges [30].

3.1.3. Organic Matter

Permanganate oxidizability, a measure of the concentration of organic matter in water, increased from 3.84 ± 0.08 mg O_2 /L in the sowing stage to 4.64 ± 0.1 mg O_2 /L in the true leaf stage. This upward trend reflects the accumulation of dissolved organic compounds as the system operates over time. While high organic loads can influence oxygen demand, it is essential to maintain oxygen levels sufficient to support both microbial nitrification and root respiration. In recirculating systems, permanganate oxidizability values below 5 mg O_2 /L are generally monitored to ensure organic loads remain within a range manageable by the system's biological filtration [31].

In conclusion, the studied aquaponics system exhibits robust nitrification. The transition from the seeding to the true leaf stage was characterized by a slight increase in organic matter and nitrites, signaling an active biological cycle. While bulk water nutrient concentrations in this system are lower than those typical of soil-based systems with high NPK fertilization, the constant availability of water and dissolved nutrients in aquaponics is often associated in the literature with effective mineral assimilation in red amaranth. Furthermore, existing studies suggest that the plant performs well in media-based systems, demonstrating tolerance to the nutrient dynamics characteristic of aquaponic environments [37,38].

3.2. Microbiological Analyses of Water from the Aquaponic System

The microbiological analysis of the water to assess the sanitary condition and potential risks from the two recirculation system tanks (S1 and S2) and Bed 1 (S1/B1) is presented in Table 2 and shows the presence of microbial load typical of an aquatic environment with organic matter and active biological processes.

Table 2. Microbiological indicators of water from the aquaponic recirculating system (S1, S2, and S1/B1).

№	Parameter	Method	Unit	S1	S2	S1/B1
				(Tank 1)	(Tank 2)	(Tank1/Bed1)
1.	Total mesophilic microorganisms (37 °C)	Serial dilution + direct plating, 1 mL	CFU/mL	6.08×10^2	7.68×10^2	7.60×10^2
2.	Total coliforms	Vacuum filtration	CFU/mL	1.06×10^4	1.17×10^4	1.43×10^4
3	<i>Escherichia coli</i>	Vacuum filtration	CFU/mL	0	0	0
4	<i>Enterococci</i>	Vacuum filtration	CFU/mL	0.89×10^2	0.86×10^2	1.01×10^2

The microbiological analysis data (Table 2) revealed the complete absence of *E. coli* in all examined samples (0 CFU/mL), which constitutes a clear indicator of the absence of fecal contamination and demonstrates effective sanitary control within the system. Similar observations were reported by Nissen et al. [39], who identified a low sanitary risk in well-managed aquaponic systems.

The highest values were observed for coliform bacteria, with counts of 1.06×10^4 CFU/mL (S1), 1.17×10^4 CFU/mL (S2), and 1.43×10^4 CFU/mL (S1/B1). The elevated concentration in the plant bed is expected and may be attributed to the accumulation of organic matter and the development of microbial consortia typical of aquaponic environments. These findings are consistent with the observations of Nissen et al. [39], who linked microbial diversity in aquaponic systems to plant surface colonization and organic loading.

The total mesophilic microorganism counts ranged within a relatively narrow interval: 6.08×10^2 CFU/mL (S1), 7.68×10^2 CFU/mL (S2), and 7.60×10^2 CFU/mL (S1/B1). These levels of microbial load reflect a stable and active microbial community, characteristic of functioning recirculating systems. The slightly higher contamination observed in S2 and in the plant bed suggests more intensive microbial processes, likely associated with biofiltration and the mineralization of organic matter.

Enterococci were detected at significantly lower concentrations: 0.89×10^2 CFU/mL (S1), 0.86×10^2 CFU/mL (S2), and 1.01×10^2 CFU/mL (S1/B1). Their low abundance indicates limited organic contamination and an acceptable sanitary risk level. These microorganisms are considered indicators of fecal and organic pollution; therefore, their controlled presence is relevant for assessing system safety.

In summary, the absence of *E. coli*, combined with moderate levels of mesophilic bacteria and elevated, yet expected, coliform counts, indicates a well-functioning aquaponic system with an active yet controlled microbiota. Nevertheless, the recorded values highlight the necessity for regular microbiological monitoring and the maintenance of optimal hygienic conditions to minimize potential risks.

3.3. Chemical Composition of the Leaf Biomass

3.3.1. Chemical Composition, Ash Content, and Mineral Profile

The data obtained from the chemical analysis of dried red amaranth leaf biomass are presented in Table 3. The obtained results were compared with literature data, with all parameters expressed on a dry matter basis to ensure accurate comparison.

Table 3. Results of the chemical analysis of red amaranth (*A. cruentus*) leaves.

Nº	Type of Test/Characteristic	Unit of Measurement	Test Result (Mean $\pm$ SD)	Test Conditions
1	Moisture *	% w/w	6.70 ± 0.17	(21.3 °C $\pm$ 0.2) °C (35 $\pm$ 3)% rH
2	Dry Matter *	% w/w	93.3 ± 1.1	(21.3 °C $\pm$ 0.2) °C (35 $\pm$ 3)% rH
3	Crude Protein	% w/w	23.69 ± 0.23	(21.3 °C $\pm$ 0.2) °C (35 $\pm$ 3)% rH
4	Lipids	% w/w	2.19 ± 0.11	(21.3 °C $\pm$ 0.2) °C (35 $\pm$ 3)% rH
5	Nitrogen-Free Extract (NFE)	% w/w	42.0 ± 0.8	(21.3 °C $\pm$ 0.2) °C (35 $\pm$ 3)% rH
6	Crude Fiber	% w/w	2.85 ± 0.13	(21.3 °C $\pm$ 0.2) °C (35 $\pm$ 3)% rH
7	Ash	% w/w	22.6 ± 0.3	(21.3 °C $\pm$ 0.2) °C (35 $\pm$ 3)% rH

* The moisture and dry matter contents are determined in order to calculate the quantities of the other substances.

3.3.2. Crude Protein

The determined crude protein content of $23.69 \pm 0.23\%$ in the leaves of *A. cruentus* cultivated in a closed-loop aquaponic system under controlled conditions was comparable to, or higher than, the values reported for soil-based and greenhouse cultivation systems. Under conventional cultivation conditions, Ngugi et al. [40] and Stevanović et al. [41] reported protein levels for red amaranth within the range of 18–20%, whereas Torres et

al. [42] documented higher values for the 'Elena' ($\approx 23\%$) and 'Aurelia' ($\approx 21\%$) cultivars grown under field and greenhouse conditions.

The broader range of 12–29% reported by Andini et al. [43] across different amaranth species and genotypes further emphasizes the substantial influence of cultivation conditions on protein accumulation. Very similar to the present findings are the data reported by Fasuyi [44], indicating a crude protein content of 23% in soil-grown *A. cruentus* cultivated in Northwestern Nigeria. Ziena [45] reported crude protein concentrations in the leaf biomass of red amaranth ranging from 25.46 to 29.02% under conventional cultivation conditions with three different nitrogen fertilization rates in Egypt.

In this context, the results of the present study demonstrate that the aquaponic system provides conditions enabling the attainment of protein levels comparable to the highest values reported under conventional cultivation practices. The elevated crude protein content in the leaves of *A. cruentus* highlights its high nutritional value and superiority compared with several traditionally consumed leafy vegetables. For comparison, dehydrated spinach exhibited a protein content of approximately 8.2% [46], which is substantially lower than the values established for red amaranth in the present investigation. According to Sarker and Oba [47], the continuous availability of assimilable nitrogen-containing compounds and the stable microclimatic conditions in aquaponic systems favor protein biosynthesis, resulting in protein levels comparable to or exceeding those observed under soil-based and greenhouse cultivation systems.

3.3.3. Crude Fat/Lipids

The crude fat content determined in the leaf biomass of *A. cruentus* was $2.19 \pm 0.11\%$, which is consistent with the nutritional composition characteristic of the species. The lipid fraction of amaranth is distinguished by a high proportion of polyunsaturated fatty acids (PUFAs), predominantly linoleic and α -linolenic acids. These compounds have attracted considerable scientific interest due to their cardioprotective properties and their role in the regulation of metabolic health [48,49].

In the context of the present study, the recorded crude fat content ($2.19 \pm 0.11\%$) in *A. cruentus* cultivated in an aquaponic system is comparable to the results reported by Sarker and Oba [47] for *Amaranthus tricolor* L. cultivated hydroponically as microgreens, where the crude fat content was approximately 2.5%. This comparison highlights the similar lipid profile of amaranth cultivated in controlled soilless systems, irrespective of differences in species and developmental stage. In contrast, under conventional soil cultivation, Fasuyi et al. [50] reported substantially higher crude fat levels in dehydrated leaves of *A. cruentus*, reaching up to 5.4% of dry matter. This variability supports the hypothesis that specific environmental conditions and nutrient availability modulate lipid biosynthesis [36]. Previous studies have shown that soilless cultivation systems may influence plant metabolic activity and the accumulation of nutritionally important compounds compared with conventional cultivation practices [51,52].

3.3.4. Carbohydrates/Nitrogen-Free Extract (NFE)

The obtained value for nitrogen-free extract (NFE) in the present study (42% DW) is comparable to published data for *A. cruentus* cultivated under conventional conditions. Fasuyi [44] reported an NFE content of approximately 43–43.5% in the leaves of red amaranth cultivated in soil, indicating that the carbohydrate fraction represents a significant component of the chemical composition of the leaf biomass. Similar results were also documented by Ziena [45], who observed high NFE values (approximately 44–45%) in conventionally cultivated red amaranth, further confirming the stability of carbohydrate content regardless of cultivation conditions.

In a more recent study, Managa and Nemadodzi [36] compared the chemical composition of leafy amaranth cultivated conventionally in soil and under controlled greenhouse conditions and found that NFE values remained within a similar range between the two systems despite the observed differences in protein content. This observation indicates that cultivation conditions exert a stronger influence on the protein fraction, whereas the carbohydrate component remains relatively stable.

In this context, the results of the present study demonstrate that the aquaponic system, characterized by continuous availability of assimilable nitrogen-containing compounds, does not lead to a reduction in NFE despite the stimulated accumulation of crude protein. Accordingly, Sarker et al. [53] reported that under intensive nitrogen nutrition, the metabolism of leafy amaranth is directed primarily toward protein biosynthesis without substantial reduction in the carbohydrate fraction. Therefore, the combination of high crude protein content and relatively high and stable NFE values ($\approx 42\%$) characterizes the nutritional profile of *A. cruentus* cultivated in an aquaponic system as balanced and comparable to that observed in conventional cultivation systems.

3.3.5. Crude Fiber Content

The crude fiber content in the leaf biomass of *A. cruentus* was $2.85 \pm 0.13\%$. This value indicates a moderate level of insoluble dietary fiber, which is characteristic of leafy amaranths. Literature data on soil-grown red amaranth demonstrate notable variability in crude fiber content, with reported values typically ranging between 3 and 5% of dry matter [54,55].

Supporting these findings, Manyelo et al. [56] reported a crude fiber content of approximately 3.2% in leaves of *A. cruentus* cultivated under conventional soil conditions. Similar levels were previously documented by Fasuyi [44] and Ziena [45], who emphasized that although fiber content is relatively stable, it is directly influenced by agro-climatic conditions and the ontogenetic developmental stage, particularly leaf maturity.

From a nutritional perspective, crude fiber is a critical component of leafy vegetables, playing an essential role in improving gastrointestinal function and in the prevention of metabolic disorders. According to Sarker et al. [53] and Rastogi and Shukla [57], the high functional value of leafy amaranth is determined by a precise balance among dietary fiber, plant proteins, and a rich mineral profile.

The comparatively lower crude fiber levels observed under aquaponic cultivation are likely attributable to the specific hydroponic-like growth regime, which promotes more intensive vegetative growth. This results in the formation of more tender leaf tissues with a lower degree of lignification compared to plants grown under field conditions. Consequently, the leaf biomass of *A. cruentus* cultivated under aquaponic conditions retains high nutritional and functional value, further confirming its significance as a health-promoting leafy vegetable.

3.3.6. Ash Content and Mineral Composition

Ash content is the inorganic mineral residue obtained after complete combustion of organic matter and serves as an indicator of total mineral content, including potassium, calcium, phosphorus, magnesium, iron, and other elements, which are of essential importance for nutritional value.

Within the context of the present study, the ash content of 22.57% recorded in the leaf biomass of *A. cruentus* cultivated in an aquaponic system is consistent with the high mineral levels commonly reported for leafy vegetables. Ash content is considered an indicator of total mineral accumulation and reflects the plant's capacity to absorb and retain macro- and micronutrients in leaf tissues [58]. Under aquaponic conditions, this

accumulation may be enhanced by the continuous availability and high bioavailability of dissolved mineral ions.

Comparable high values have been reported by Ziena [45] for soil-grown *A. cruentus*, where ash content ranged between 19.78 and 22.71%. This indicates that red amaranth is inherently characterized by high mineral density, regardless of the production system employed. Similar results have been reported for other leafy amaranth species. Nwaogu et al. [59] reported high ash content in leaves of soil-grown *Amaranthus hybridus* L., which the authors associated with active mineral metabolism and elevated concentrations of Ca, Mg, and K. In the study by Yiblet and Adamu [60], which evaluated the nutritional and mineral composition of several wild edible plants from northwestern Ethiopia, *Amaranthus graecizans* L. exhibited among the highest ash contents ($24.70 \pm 0.15\%$), indicating substantial accumulation of mineral components in leaf biomass. This variability is attributed to both genotypic differences and environmental and mineral nutrition conditions.

The higher ash content observed in the present study suggests that the aquaponic system provides a stable and balanced supply of nutrients, thereby promoting the accumulation of inorganic constituents in leaf biomass. In this context, Sarker et al. [53] noted that under controlled systems with continuous nutrient availability, leafy amaranth exhibits enhanced mineral accumulation capacity without compromising other nutritional fractions. The obtained results confirm that the ash fraction constitutes a significant and stable component of the nutritional profile of red amaranth, and that aquaponic cultivation enables mineral densities comparable to or higher than those reported in conventional soil-based systems.

The mineral composition of the red amaranth leaves analyzed in the present study is characterized by a high content of macro- and micronutrients, indicating substantial mineral density and nutritional value (Figures 2 and 3). For accurate comparison and interpretation, all values were standardized on a dry weight basis (mg/g).

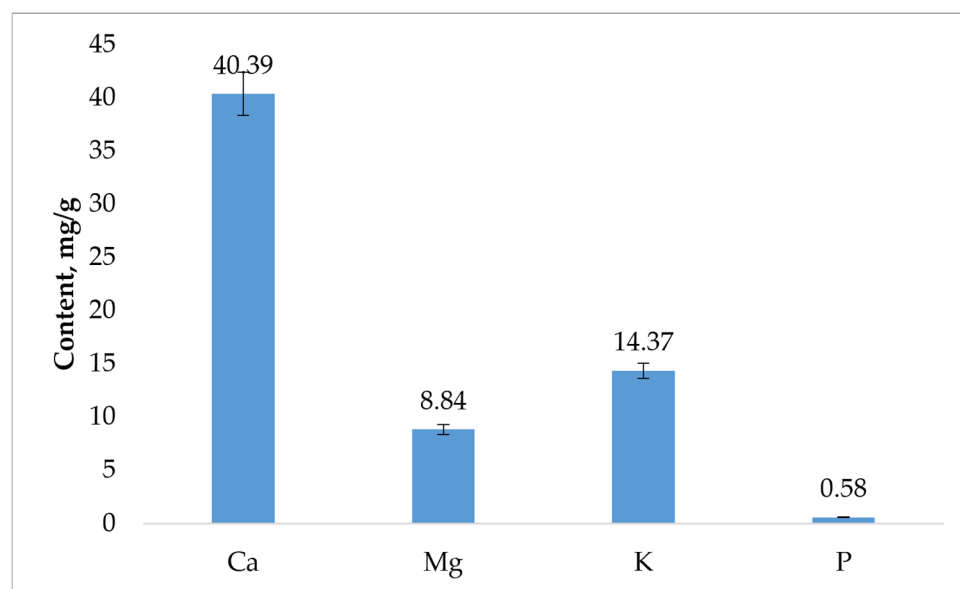


Figure 2. Macronutrient content in the leaf biomass of red amaranth (*A. cruentus*).

The Ca concentration in the leaves of *A. cruentus* in the present study reached 40.39 mg/g, indicating substantial accumulation of this macronutrient under aquaponic cultivation conditions. In the literature, lower Ca values are reported for soil-grown *A. cruentus*, typically ranging between 18.9 and 30.9 mg/g [36], with variations attributed to soil properties and fertilization regimes. The elevated Ca content observed in the present

study suggests that the controlled mineral composition of the aquaponic water environment enhances the uptake and accumulation of this element. Calcium is essential for the structural stability of cell walls and membranes, as well as for the regulation of numerous physiological processes, which underlines its importance for leaf biomass quality.

An interesting physiological relationship is observed between relatively low crude fiber content and elevated Ca levels. In general, Ca contributes to cell wall strengthening through the formation of calcium pectates, resulting in increased structural rigidity. However, under aquaponic cultivation conditions, intensive vegetative growth and a shorter tissue differentiation period lead to reduced lignification and, consequently, lower fiber content. It is likely that a portion of calcium is accumulated in vacuoles in the form of oxalates or free ions rather than being fully incorporated into structural cell wall components. This explains why leaves of red amaranth grown in aquaponic systems maintain a tender texture despite their high mineral content. A similar functional balance between mineral composition and structural properties has been reported by Rastogi and Shukla [57] in leafy vegetables.

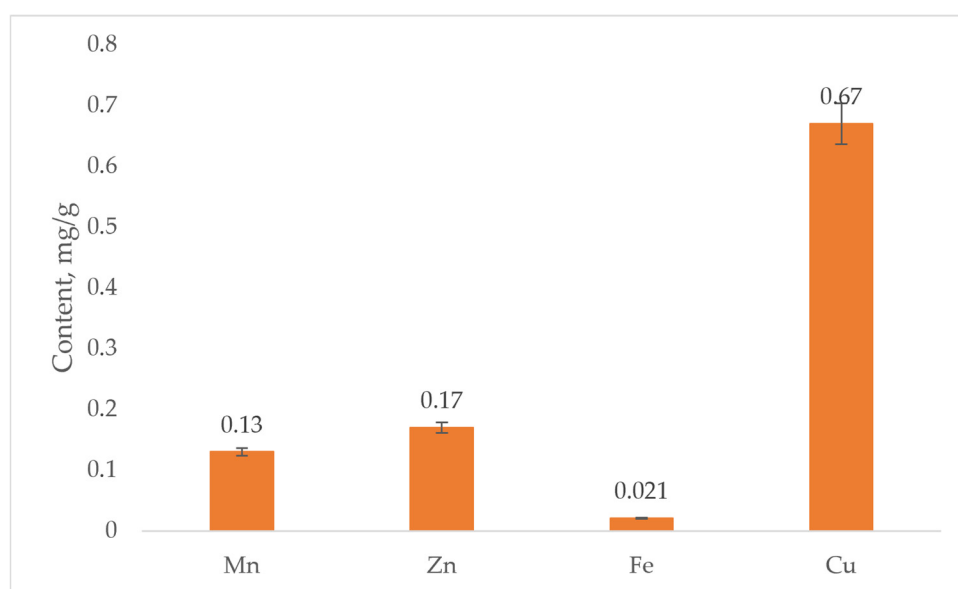


Figure 3. Content of micronutrients in the leaf biomass of red amaranth (*A. cruentus*).

The Mg concentration in the leaf biomass of *A. cruentus* was 8.84 mg/g, which is comparable to values reported for leafy amaranths cultivated under different environmental conditions. Managa and Nemadodzi [36] reported Mg concentrations between 2.5 and 6.2 mg/g in soil-grown *Amaranthus* accessions, while Ziena [45] documented values ranging from 9.88 to 12.49 mg/g dry weight in *A. cruentus* leaves. Additional studies on leafy amaranths by Nwaogu et al. [59] also demonstrated considerable variation in mineral composition depending on species and cultivation conditions. The relatively high Mg content observed in the present study may be associated with the continuous availability and high bioavailability of magnesium ions in the aquaponic environment, as well as with specific nutritional and microclimatic conditions.

The K content determined in the leaves of *A. cruentus* was 14.37 mg/g DW. Potassium is the dominant macronutrient in amaranth leaf biomass and plays a central role in osmotic regulation, assimilate transport, and enzyme activation. Sarker et al. [61] reported potassium concentrations ranging from 6.62 to 12.41 mg/g DW in leafy amaranth genotypes cultivated under field conditions. The value obtained in the present study

slightly exceeds the upper range of these reported values, indicating that aquaponic systems may provide favorable conditions for potassium accumulation. From a nutritional perspective, high K content is important for maintaining electrolyte balance and supporting normal nerve and muscle function in humans.

The P content in the leaf biomass of *A. cruentus* was 0.58% DW, which is consistent with previously reported phosphorus concentrations in leafy amaranths cultivated under conventional conditions [62]. Phosphorus plays a fundamental role in cellular energy transfer, nucleic acid synthesis, membrane phospholipid formation, and overall plant growth and development. The concentration observed in the present study indicates adequate phosphorus availability and efficient nutrient uptake under aquaponic cultivation conditions.

In plants, the uptake of P and nitrogen N is tightly interconnected and regulated by the availability of nutrients in the growth medium. Phosphorus is essential for energy transfer processes, particularly through its role in ATP-dependent reactions that drive protein synthesis and overall cellular metabolism. In aquaponic systems, the continuous supply of dissolved nutrients enhances nutrient uptake efficiency, thereby promoting improved plant growth and metabolic activity [63].

The average iron content in leaf biomass was found to be the lowest among the microelements—0.021 mg/g DW (Figure 3), followed by manganese—0.13 mg/g, which falls within the intermediate range of values reported for *A. cruentus* (0.08–0.18 mg/g) by Sarker et al. [61], under soil cultivation conditions. Manganese plays a key role in photosynthetic processes, carbohydrate and nitrogen metabolism, and contributes significantly to the antioxidant capacity of plants. The present results confirm the stable accumulation of this micronutrient under aquaponic cultivation conditions. Elevated Mg concentrations are associated not only with chlorophyll structure but also with the activation of ribosomes and enzyme systems involved in protein synthesis. Stable Mn levels further complement these processes by supporting the antioxidant defense mechanisms of rapidly developing leaf biomass [49,61].

The Zn concentration in the leaves of *A. cruentus* was 0.17 mg/g. Zinc accumulation in amaranth species is known to vary depending on genotype, soil composition, and cultivation conditions. Field-grown *Amaranthus* has been reported to contain approximately 50–60 mg/kg DW (0.05–0.06 mg/g), with considerable variability across studies and species [54,61].

In comparison, higher or more variable micronutrient uptake may occur under fertilized or stress-influenced conditions, while controlled cultivation systems tend to produce more regulated elemental concentrations. These findings indicate that Zn accumulation in amaranth is strongly influenced by the growth environment and nutrient availability.

The recorded Cu concentration was 0.67 mg/g. Copper accumulation in *A. cruentus* and related leafy vegetables is known to be influenced by environmental and agronomic factors, including soil composition, nutrient availability, and exposure to metal sources. Previous studies and meta-analytical evidence indicate that heavy metal uptake in *Amaranthus* species can vary considerably depending on cultivation conditions and species-specific physiological responses [64,65]. In general, field-grown systems tend to show higher variability in micronutrient accumulation compared to controlled cultivation systems such as hydroponics or aquaponics, where nutrient supply is more regulated. Copper plays an essential role in plant metabolism as a cofactor in enzymes involved in photosynthesis and antioxidant defense, and its accumulation reflects both environmental availability and physiological regulation.

Overall, the results on mineral composition demonstrate that *A. cruentus* cultivated in an aquaponic system is characterized by high and well-balanced concentrations of

macro- and micronutrients, frequently reaching or exceeding levels reported for soil- and hydroponic-cultivation systems. Adequate Cu and Zn concentrations support the proper functioning of electron transport chains and enzymatic systems associated with photosynthesis. Furthermore, balanced micronutrient availability prevents the reduction in the carbohydrate fraction even under intensified nitrogen metabolism and enhanced protein synthesis, thereby supporting the concept proposed by Managa and Nemadodzi [36] regarding the pronounced metabolic plasticity of amaranth.

3.3.7. Determination of Total Phenolic Content (TPC)

The TPC of aquaponically grown red amaranth in the present study was 5.08 mg GAE/g DW, indicating a moderate phenolic content. This value is consistent with previous reports for *A. cruentus*, which describe abundant hydroxycinnamic and hydroxybenzoic acids, as well as flavonoids such as rutin and quercetin [66]. The presence of betalains, which contain phenolic and cyclic -NH₂ groups, likely contributed substantially to the measured TPC. In addition, these findings demonstrate that aquaponic cultivation supports the biosynthesis of nitrogen-containing pigments and other phenolic compounds at levels comparable to hydroponic and soil-grown systems. Such results highlight the potential of aquaponic amaranth as a functional food with high antioxidant activity [67].

3.3.8. Determination of Total Flavonoid Content (TFC)

The registered TFC of 5.07 mg QE/g DW approaches the upper range reported for *A. cruentus* leaf extracts (5.2 mg QE/g DW) in scientific literature [68]. The latter proposes that the aquaponic system effectively supports the metabolic pathways responsible for flavonoid biosynthesis. The similarity between TPC and TFC values indicates that flavonoids constitute a substantial portion of the total phenolic fraction.

3.3.9. Betalain, Anthocyanin (TAntC), and Chlorophyll Content

As expected for *A. cruentus*, anthocyanins were not detected due to the taxonomic replacement of anthocyanins with betalains in Caryophyllales species [69,70]. The determined betalain content of 3.73 mg/g DW is moderate and reflects the stability of these nitrogen-containing pigments under aquaponic conditions. The presence of betalains demonstrates that the aquaponic system provides adequate micronutrients and environmental conditions to support secondary metabolite biosynthesis. These results are consistent with previously published literature data indicating that leaf coloration in red amaranth is primarily associated with the presence of betalains rather than anthocyanins [71].

The measured total chlorophyll content (5.35 mg/g DW) approximates the upper range reported for amaranth leaves (4–8 mg/g DW) in scientific literature [72]. The experimental data established that chlorophyll a was the dominant pigment, suggesting efficient photosynthetic performance in the aquaponic system. The absence or detection of low chlorophyll b levels may result from the selective extraction of ethanol or the specific light spectrum provided in the aquaponic environment. Overall, high chlorophyll a content combined with stable betalain levels indicates minimal metabolic stress, confirming the effectiveness of the aquaponic system for plant growth and pigment accumulation. Chlorophyll a is essential for photosynthesis and high levels indicate robust photosynthetic activity, which is generally reduced when plants are under severe abiotic stress, such as drought or high salinity, which causes pigment degradation. On the other side, betalains (red-violet betacyanins and yellow-orange betaxanthins) are nitrogen-containing pigments that function as antioxidants to protect plant tissues from reactive oxygen species (ROS) caused by stress [73]. Under metabolic stress, betalain

synthesis increases dramatically as a defense mechanism. However, stable betalain levels are indicative of the absence of additional constraining of the plant to utilize these secondary metabolites, signifying the lack of oxidative stress. [53,74,75]. Consequently, the observed combination indicates that the plant could be characterized by high photosynthetic vitality associated with high chlorophyll content and the absence of damaging metabolic stress due to the stable betalain level [76].

When compared to hydroponic and soil-grown *A. cruentus*, aquaponically grown plants exhibit comparable TPC and TFC levels, demonstrating that controlled soilless systems are capable of producing red amaranth with equivalent or superior bioactive potential. The experimentally determined betalain profile further confirms that aquaponic cultivation supports the synthesis of nitrogen-containing pigments, as typically observed in soil-grown plants. Figure 4 summarizes and visually demonstrates that aquaponically grown red amaranth accumulates phenolics, flavonoids, betalains, and chlorophyll at levels comparable to hydroponic and soil-grown species, confirming its potential as a functional food with high antioxidant and pigment content.

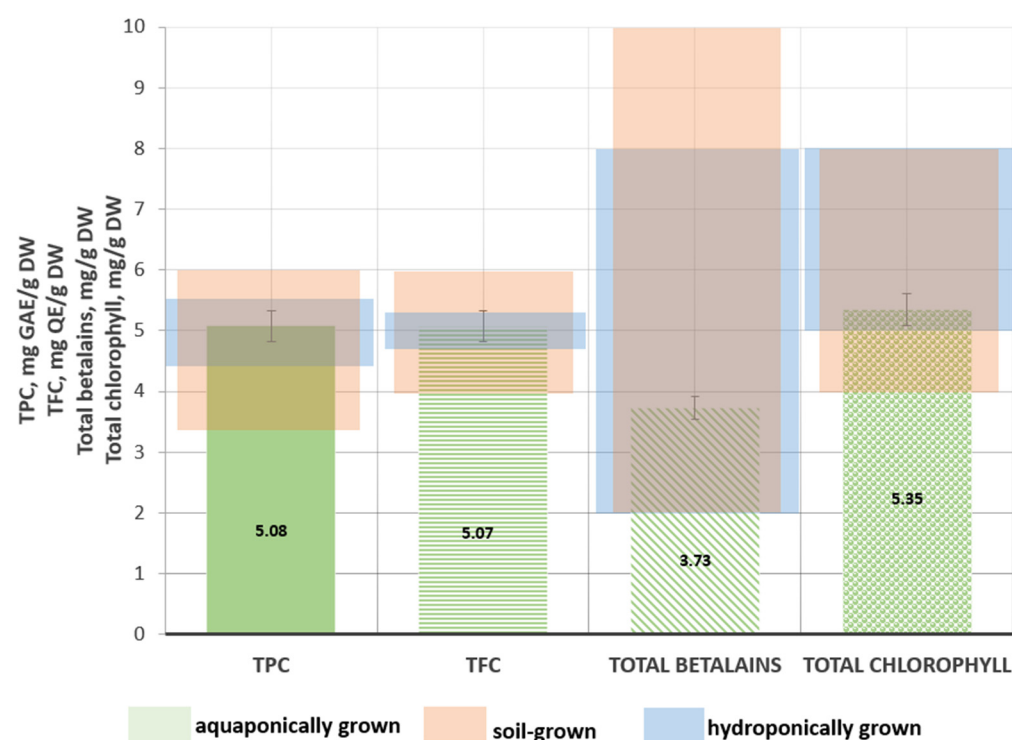


Figure 4. Comparative phytochemical and pigment content of red amaranth leaves under different cultivation systems—aquaponics (this study), hydroponic, and soil [68,69,77].

3.3.10. Radical-Scavenging Potential

The conducted spectrophotometric investigation of the DPPH, ABTS, and FRAP radical-scavenging potential of ethanol extract of aquaponically grown red amaranth provided quantitative evidence of its high biological activity. The comparative analysis of the obtained data revealed heterogeneity in the antioxidant response of the applied assays as well as differences in the reactivity of the phytochemical constituents present in the sample (Table 4).

ABTS capacity: The inhibition value of 89.27% in the ABTS assay determined in the present study indicates an exceptionally high overall radical-scavenging capacity. The high reactivity of red amaranth in this system can probably be attributed to the synergistic interaction between betacyanins and hydrophilic phenolic acids such as ferulic and caffeic

acids. According to the fundamental study of Sarker and Oba [62], and substantiated by Prior et al. [78], these compounds predominantly operate via a single-electron transfer (SET) mechanism, rapidly reducing the blue-green chromophore of the ABTS radical to its colorless form. The high inhibition value, therefore, reflects a substantial concentration of low-molecular-weight antioxidants in the ethanol extract. The registered ABTS capacity exceeds or approaches the upper limit reported for hydroponically cultivated *Amaranthus* species and falls within the higher spectrum of values reported for soil-grown plants. This elevated ABTS response suggests a particularly high electron-transfer antioxidant capacity in aquaponically cultivated red amaranth, likely reflecting enhanced accumulation of hydrophilic phenolic compounds and betalain pigments [13,79–81].

Table 4. Comparative antioxidant activity (DPPH, ABTS, and FRAP assays) of red amaranth grown under aquaponic conditions in the present study and values reported for hydroponic and soil-grown plants in the literature.

Cultivation System	Species	DPPH, %	ABTS, %	FRAP, %	Ref.
Aquaponic (this study)	<i>Amaranthus cruentus</i>	58.99 ± 2.94	89.27 ± 4.02	4.31 ± 0.21	This study
Hydroponic cultivation	<i>Amaranthus tricolor</i> /leafy amaranth	35–70 *	60–80 *	3–6 *	[13,79]
Soil cultivation	<i>Amaranthus cruentus</i>	20–60 *	50–90 *	3–7 *	[80,81]
	<i>Amaranthus</i> spp.	30–65 *	50–78 *	n/a	[13,61,79]
	<i>Amaranthus cruentus</i>	50 (IC ₅₀ = 75.6 µg/mL)	n/a	4–6 *	[80]

* Values from the literature originally reported in Trolox equivalents, ascorbic acid equivalents, or IC₅₀ units were approximated to percentage inhibition ranges based on typical assay responses reported for *Amaranthus* extracts under comparable experimental conditions.

DPPH Activity: The inhibition value of 58.99% in the DPPH assay registered in the current investigation is particularly significant. Unlike ABTS, DPPH is a stable free radical that often exhibits lower sensitivity toward plant extracts due to its sterically hindered molecular configuration. Nevertheless, the relatively high inhibition observed in this system confirms that red amaranth is rich in compounds capable of acting as efficient hydrogen atom donors through a hydrogen atom transfer (HAT) mechanism. Betalains play a central role in this process. by Karamać et al. [82]. Bastos and Schliemann [83] demonstrated that, in contrast to common anthocyanins, betalains present in species of the genus *Amaranthus* display higher radical-scavenging capacity in polar protic solvents such as ethanol. This property explains the ability of the extract to neutralize more than half of the available DPPH radicals during the standard incubation period. A number of scientific studies emphasized that betalains, particularly amaranthin, possess structural features that confer remarkable resonance stabilization of radical intermediates [83,84]. Betalains, including amaranthin (a major pigment in *Amaranthus* species), achieve remarkable radical stabilization through a highly conjugated 1,7-diazaheptamethinium system. This structure allows for extensive delocalization of unpaired electrons, making betalains exceptionally effective antioxidants that often surpass ascorbic acid in radical-scavenging capacity [85]. As a result, these pigments may exhibit higher efficiency than many anthocyanins in neutralizing lipophilic radicals [74,86].

Similarly to the results observed in the present study, Paško et al. [87] investigated ethanol extracts from leafy vegetables and concluded that such extracts frequently exhibit a hydrogen atom transfer (HAT) mechanism in interactions with DPPH radicals, whereas the ABTS assay is more sensitive to single-electron transfer (SET) processes [87]. This difference provides a plausible explanation for the heterogeneity observed in the results (89.27% vs. 58.99%) (Table 4).

In addition, the experimentally determined DPPH potential is consistent with the upper range of activities reported for hydroponically grown amaranth and is comparable to many soil-grown samples reported in previous studies. The result suggests that the hydrogen-atom-donating capacity of the phytochemical constituents in the aquaponic plants is similar to that observed in other cultivation systems, although slightly elevated relative to several field-grown populations [13,79].

FRAP Analysis: The FRAP assay measures the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} under acidic conditions. The obtained experimental data suggest that the antioxidant mechanism of red amaranth is primarily directed toward direct free-radical scavenging, whereas its role as a metal-reducing agent is secondary. The relatively low inhibition value obtained in the FRAP assay (4.31%) can be interpreted from the viewpoint of the findings reported by Jiménez-Aguilar and Grusak [55], who proposed that the Fe-reducing capacity of species within the genus *Amaranthus* might be masked by the presence of oxalates or other antinutritional compounds capable of chelating metal ions [55]. Such chelation reduces the amount of free Fe available to participate in the FRAP reaction, regardless of the presence of antioxidant compounds. As reported by Sarker et al. [77], betalains extracted with ethanol may exhibit structural instability at low pH values, which can lead to underestimated FRAP results [77]. In addition to betalains, flavonoid glycosides also contribute to the overall antioxidant capacity. Nana et al. [80] identified rutin and quercetin as major flavonoids in amaranth leaves. These compounds act synergistically with phenolic acids, enhancing the total radical-scavenging potential observed in the present study. The observed pattern of lower FRAP activity is consistent with trends reported in the literature for *Amaranthus* species, where the ferric-reducing antioxidant power is frequently lower than the radical-neutralization capacity measured by ABTS or DPPH.

Overall, the comparative data indicate that aquaponically cultivated red amaranth exhibits antioxidant properties that are at least comparable, and in some cases superior, to those reported for hydroponic and soil-grown plants. The particularly strong ABTS response suggests that aquaponic cultivation conditions may promote the accumulation of electron-donating phytochemicals, reinforcing the potential of this cultivation system for producing leafy vegetables with enhanced functional and nutraceutical value [80,88,89].

3.4. Microclimate

The recorded data indicated a relatively stable microclimate with a distinct diurnal dynamic typical of closed aquaponic systems. Maintaining these environmental parameters provided favorable conditions for plant growth and stable operation of the integrated aquaponic system.

The temperature and humidity conditions maintained during the experimental period were within the ranges reported as suitable for amaranth cultivation under controlled environments.

Gudžinskaitė et al. [90] reported that cultivation of *Amaranthus tricolor* under comparable temperature conditions did not result in significant growth limitations during the early developmental stages.

Similarly, Carrasco et al. [30] demonstrated that substrate selection significantly influenced the growth and nutritional composition of *A. cruentus* microgreens cultivated in a floating hydroponic system under average daytime temperatures of 25 ± 3 °C, nighttime temperatures of 17 ± 2 °C, and relative humidity ranging from 60 to 75%.

Literature data for *A. tricolor* further indicate that temperatures between 25 and 30 °C are generally associated with more intensive growth and enhanced metabolic activity, while the species exhibits relatively high tolerance within the broader range of 20–35 °C.

Temperatures exceeding 35–40 °C may induce physiological stress associated with activation of antioxidant defense systems and increased accumulation of secondary metabolites, although such stress conditions may negatively affect productivity [91].

4. Conclusions

This study demonstrates that recirculating aquaponic systems provide an adequate and resource-efficient environment for the cultivation of Red Amaranth (*A. cruentus* L.). The robust biological filtration maintained highly efficient nitrification throughout the growth cycle, successfully keeping toxic nitrogen species below hazardous thresholds while ensuring continuous nutrient assimilation. This steady, bioavailable nutrient stream triggered an enhanced secondary metabolic response in the plants, resulting in high-quality leaf biomass. In particular, high levels of crude protein, Ca, Mg, and K, as well as phenolic compounds and betalains, were observed. Furthermore, systematic microbiological testing verified strict safety compliance and negligible pre-harvest contamination risks. Collectively, these findings establish integrated aquaponics as a highly viable, scalable, and circular agricultural framework capable of commercializing premium functional foods while decoupling intensive vegetable production from soil dependency and synthetic fertilizers. While simultaneous comparative trials with field and standalone hydroponic treatments would be required to statistically validate relative performance advantages, the current observations firmly highlight the capacity of this system to optimize key nutritional and bioactive attributes in amaranth.

Author Contributions: Conceptualization, N.G. and Z.Y.; methodology, Z.Y., G.B. and M.T.; investigation, D.G., S.H., S.T., R.M., G.G. and N.T.; formal analysis, D.G., S.H. and Z.Y.; data curation, D.G., S.H. and Z.Y.; visualization, S.T., Z.Y. and M.T.; writing—original draft preparation, D.G., S.H., Z.Y., N.G., M.T., G.B., D.T.S., S.T., R.M. and G.G.; writing—review and editing, Z.Y., N.G., M.T. and G.B.; supervision, N.G.; project administration, N.G.; funding acquisition, N.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by project BG16RFPR002-1.014-0012-C01, “Establishment and sustainable development of a Center of Competence ‘AgriFood Systems and Bioeconomy’”, financed by the European Regional Development Fund through the “Program for Research, Innovation and Digitalisation for Smart Transformation” (PRIDST) and by the Bulgarian National Recovery and Resilience Plan, Component “Innovative Bulgaria”, through project BG-RRP-2.004-0006-C03, “Development of scientific research and innovation at Trakia University in the service of health and sustainable well-being”.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: During the preparation of this work, the authors used COPILOT (<https://copilot.microsoft.com/>; Microsoft Corporation, Redmond, WA, USA) in order to improve the language and presentation of the manuscript. No fixed version number is available for this tool. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Conflicts of Interest: Author Neven Terziev was employed by the company Aqua Field Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Foley, J.A.; Ramankutty, N.; Brauman, K.A.; Cassidy, E.S.; Gerber, J.S.; Johnston, M.; Mueller, N.D.; O’Connell, C.; Ray, D.K.; West, P.C.; et al. Solutions for a cultivated planet. *Nature* **2011**, *478*, 337–342. <https://doi.org/10.1038/nature10452>.

2. FAO. AQUASTAT Main Database. Food and Agriculture Organization of the United Nations. 2020. Available online: <https://www.fao.org/aquastat/en/> (accessed on 14 April 2026).
3. Tilman, D.; Balzer, C.; Hill, J.; Befort, B.L. Global food demand and the sustainable intensification of agriculture. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 20260–20264. <https://doi.org/10.1073/pnas.1116437108>.
4. Rockström, J.; Steffen, W.; Noone, K.; Persson, A.; Lambin, E.F.; Lenton, T.M. A safe operating space for humanity. *Nature* **2009**, *461*, 472–475. <https://doi.org/10.1038/461472a>.
5. European Commission. *A Farm to Fork Strategy for a Fair, Healthy and Environmentally-Friendly Food System*; European Commission Document Brussels: Brussel, Belgium, 2020.
6. Junge, R.; König, B.; Villarreal, M.; Komives, T.; Jijakli, H. Strategic points in aquaponics. *Water* **2017**, *9*, 182. <https://doi.org/10.3390/w9030182>.
7. Goddek, S.; Espinal, C.A.; Delaide, B.; Jijakli, H.; Schmautz, Z.; Wuertz, S.; Keesman, K.J. Navigating towards decoupled aquaponic systems: A system dynamics design approach. *Water* **2016**, *8*, 303. <https://doi.org/10.3390/w8070303>.
8. Somerville, C.; Cohen, M.; Pantanella, E.; Stankus, A.; Lovatelli, A. *Small-Scale Aquaponic Food Production: Integrated Fish and Plant Farming*; FAO Fisheries and Aquaculture Technical Paper No. 589; Food and Agriculture Organization of the United Nations: Rome, Italy, 2014. Available online: <https://openknowledge.fao.org/handle/20.500.14283/i4021e> (accessed on 14 April 2026).
9. Love, D.C.; Fry, J.P.; Genello, L.; Hill, E.S.; Frederick, J.A.; Li, X.; Semmens, K. An international survey of aquaponics practitioners. *PLoS ONE* **2014**, *9*, e102662. <https://doi.org/10.1371/journal.pone.0102662>.
10. Babatunde, T.A.; Ibrahim, K.; Abdulkarim, B.; Wagini, N.H.; Usman, S.A. Co-production and biomass yield of amaranthus (*Amaranthus hybridus*) and tilapia (*Oreochromis niloticus*) in gravel-based substrate filter aquaponic. *Int. J. Recycl. Org. Waste Agric.* **2019**, *8*, 255–261. <https://doi.org/10.1007/s40093-019-00297-5>.
11. Gichana, Z. Phytoremediation potential of selected plants & growth of *Oreochromis niloticus* (Linnaeus, 1758) in aquaponic systems. *Asian J. Fish. Aquat. Res.* **2024**, *26*, 70–80.
12. Sarker, U.; Hossain, M.N.; Iqbal, M.A.; Oba, S. Bioactive components and radical scavenging activity in selected advance lines of salt-tolerant vegetable amaranth. *Front. Nutr.* **2020**, *7*, 587257. <https://doi.org/10.3389/fnut.2020.587257>.
13. Bang, J.-H.; Lee, K.J.; Jeong, W.T.; Han, S.; Jo, I.-H.; Choi, S.H.; Cho, H.; Hyun, T.K.; Sung, J.; Lee, J.; et al. Antioxidant Activity and Phytochemical Content of Nine *Amaranthus* Species. *Agronomy* **2021**, *11*, 1032. <https://doi.org/10.3390/agronomy11061032>.
14. Monsees, H.; Kloas, W.; Wuertz, S. Decoupled systems on trial: Eliminating bottlenecks to improve aquaponic processes. *PLoS ONE* **2017**, *12*, e0183056. <https://doi.org/10.1371/journal.pone.0183056>.
15. Meier, U. *Growth Stages of Mono- and Dicotyledonous Plants: BBCH Monograph*, 2nd ed.; Federal Biological Research Centre for Agriculture and Forestry: Bonn, Germany, 2001. <https://doi.org/10.5073/20180906-074619>.
16. *BDS ISO 6496:2000*; Animal Feeding Stuffs—Determination of Moisture and Other Volatile Matter Content. Bulgarian Institute for Standardization: Sofia, Bulgaria, 2000.
17. *BDS EN ISO 5983-1:2006*; Animal Feeding Stuffs—Determination of Nitrogen Content and Calculation of Crude Protein Content—Part 1: Kjeldahl Method (ISO 5983-1:2005). Bulgarian Institute for Standardization: Sofia, Bulgaria, 2006.
18. *BDS ISO 6492:2007*; Animal Feeding Stuffs—Determination of Fat Content. Bulgarian Institute for Standardization: Sofia, Bulgaria, 2007.
19. *BDS ISO 5984:2022*; Animal Feeding Stuffs—Determination of Crude Ash. Bulgarian Institute for Standardization: Sofia, Bulgaria, 2022.
20. Lichtenthaler, H.K.; Wellburn, A.R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591–592. <https://doi.org/10.1042/bst0110591>.
21. Yaneva, Z.; Grozeva, N.; Todorova, M.; Kamenova-Nacheva, M.; Staleva, P.; Memdueva, N.; Tzanova, M.T. Comparison of the Potential of “Green” Classical and Natural Deep Eutectic Solvents in the Production of Natural Food Colorant Extracts from the Roots of *Alkanna tinctoria* (L.). *Foods* **2025**, *14*, 584. <https://doi.org/10.3390/foods14040584>.
22. Dinev, T.; Tzanova, M.; Velichkova, K.; Dermendzhieva, D.; Beev, G. Antifungal and Antioxidant Potential of Methanolic Extracts from *Acorus calamus* L., *Chlorella vulgaris* Beijerinck, *Lemna minuta* Kunth and *Scenedesmus dimorphus* (Turpin) Kützing. *Appl. Sci.* **2021**, *11*, 4745. <https://doi.org/10.3390/app11114745>.
23. Sokolova, D.V.; Shvachko, N.A.; Mikhailova, A.S.; Popov, V.S.; Solovyeva, A.E.; Khlestkina, E.K. Characterization of Betalain Content and Antioxidant Activity Variation Dynamics in Table Beets (*Beta vulgaris* L.) with Differently Colored Roots. *Agronomy* **2024**, *14*, 999. <https://doi.org/10.3390/agronomy14050999>.

24. Dos Santos, B.A.; Silva, D.H.S.; Bolzani, V.S.; Santos, L.A.; Schemidt, T.S.; Baffa, O. Antioxidant Properties of Plant Extracts: An EPR and DFT Comparative Study of the Reaction with DPPH, TEMPOL and Spin Trap DMPO. *J. Braz. Chem. Soc.* **2009**, *20*, 1483–1492. <https://doi.org/10.1590/S0103-50532009000800015>.
25. Grozeva, N.H.; Mineva, R.; Gospodinova, G.; Georgieva, D.; Hristova, S.; Tzanova, M.; Terzieva, S.; Beev, G.; Terziev, N.; Yaneva, Z. Impact of Aquaponic Cultivation on the Nutritional, Mineral, and Antioxidant Profile of Swiss Chard (*Beta vulgaris* var. *cicla*). *Agronomy* **2026**, *16*, 822. <https://doi.org/10.3390/agronomy16080822>.
26. Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76. <https://doi.org/10.1006/abio.1996.0292>.
27. Danner, R.I.; Mankasingh, U.; Ananthawat-Jonsson, K.; Thorarinsdottir, R.I. Designing Aquaponic Production Systems towards Integration into Greenhouse Farming. *Water* **2019**, *11*, 2123. <https://doi.org/10.3390/w11102123>.
28. Sarker, S.; Hossain, M.A.; Bristy, F.S.; Islam, M.R.; Saha, J.; Kawser, A.R.; Banu, M.R. Effects of Carbon-to-Nitrogen Ratio in Biofloc System on Water Quality, Growth Performance and Haematological Parameters of Nile Tilapia (*Oreochromis niloticus*). *Aquac. Fish Fish.* **2026**, *6*, e70249. <https://doi.org/10.1002/aff.70249>.
29. Bhambri, A.; Karn, S.K.; Kumar, A. Ammonia transformation by potential consortium in the aquatic system. *Sci. Rep.* **2025**, *15*, 6066. <https://doi.org/10.1038/s41598-025-90206-w>.
30. Carrasco, G.; Rebolledo, P.; Fuentes-Peñailillo, F.; Gómez, R.; Manríquez, P. Substrate Effects on Yield and Nutritional Quality of Amaranth Microgreens in Floating-Tray Hydroponics. *Horticulturae* **2025**, *11*, 1395. <https://doi.org/10.3390/horticulturae11111395>.
31. Lindholm-Lehto, P. Water quality monitoring in recirculating aquaculture systems. *Aquac. Fish Fish.* **2023**, *3*, 113–131. <https://doi.org/10.1002/aff.2.102>.
32. Goddek, S.; Joyce, A.; Wuertz, S.; Körner, O.; Bläser, I.; Reuter, M.; Keesman, K.J. Decoupled Aquaponics Systems. In *Aquaponics Food Production Systems*; Goddek, S., Joyce, A., Kotzen, B., Burnell, G.M., Eds.; Springer: Cham, Switzerland, 2019. https://doi.org/10.1007/978-3-030-15943-6_8.
33. Heise, J.; Müller, H.; Probst, A.J.; Meckenstock, R.U. Ammonium Removal in Aquaponics Indicates Participation of *Comammox Nitrospira*. *Curr. Microbiol.* **2021**, *78*, 894–903. <https://doi.org/10.1007/s00284-021-02358-3>.
34. Khater, E.S.; Bahnasawy, A.; Ali, S.; Abbas, W.; Morsy, O.; Sabahy, A. Study on the plant and fish production in the aquaponic system as affected by different hydraulic loading rates. *Sci. Rep.* **2023**, *13*, 17505. <https://doi.org/10.1038/s41598-023-44707-1>.
35. Medina, M.; Jayachandran, K.; Bhat, M.G.; Deoraj, A. Assessing plant growth, water quality and economic effects from application of a plant-based aquafeed in a recirculating aquaponic system. *Aquacult. Int.* **2016**, *24*, 415–427. <https://doi.org/10.1007/s10499-015-9934-3>.
36. Managa, G.M.; Nemadodzi, L.E. Comparison of Agronomic Parameters and Nutritional Composition on Red and Green Amaranth Species Grown in Open Field Versus Greenhouse Environment. *Agriculture* **2023**, *13*, 685. <https://doi.org/10.3390/agriculture13030685>.
37. Jahan, F.; Bhuiyan, M.N.H.; Islam, M.J.; Ahmed, S.; Hasan, M.S.; Bashera, A.M.; Waliullah, M.; Chowdhury, A.N.; Islam, M.B.; Saha, B.K.; et al. *Amaranthus tricolor* (red amaranth), an indigenous source of nutrients, minerals, amino acids, phytochemicals, and assessment of its antibacterial activity. *J. Agric. Food Res.* **2022**, *10*, 100419. <https://doi.org/10.1016/j.jafr.2022.100419>.
38. Hoang, L.H.; Guzman De, C.C.; Cadiz, N.M.; Tran, D.H. Physiological and phytochemical responses of red amaranth (*Amaranthus tricolor* L.) and green amaranth (*Amaranthus dubius* L.) to different salinity levels. *Legume Res.* **2019**, *43*, 206–211. <https://doi.org/10.18805/LR-470>.
39. Nissen, L.; Casciano, F.; Gianotti, A. Plant Volatiles of Lettuce and Chicory Cultivated in Aquaponics Are Associated to Their Microbial Community. *Microorganisms* **2021**, *9*, 580. <https://doi.org/10.3390/microorganisms9030580>.
40. Ngugi, C.C.; Oyoo-Okoth, E.; Manyala, J.O.; Fitzsimmons, K.; Kimotho, A. Characterization of the nutritional quality of amaranth leaf protein concentrates and suitability of fish meal replacement in Nile tilapia feeds. *Aquac. Rep.* **2017**, *5*, 62–69. <https://doi.org/10.1016/j.aqrep.2017.01.003>.
41. Stevanović, A.; Popović, V.M.; Filipović, A.; Bošković, J.Z.; Pešić, V.; Marinković, T.; Stojićević, A. Phytopharmacological profile, nutritional value and amaranthine content of *Amaranthus* and their significance in medicine. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2024**, *52*, 14070. <https://doi.org/10.15835/nbha52414070>.
42. Torres, E.V.; Valencia, E.; Simsek, S.; Linares Ramírez, A.M. Nutritional composition of leaves and grains of *Amaranthus cruentus* and *Amaranthus viridis* grown under field and greenhouse conditions. *Agronomy* **2024**, *14*, 2323. <https://doi.org/10.3390/agronomy14102323>.

43. Andini, R.; Yoshida, S.; Yoshida, Y.; Ohsawa, R. *Amaranthus* genetic resources in Indonesia: Morphological and protein content assessment in comparison with worldwide amaranths. *Genet. Resour. Crop Evol.* **2013**, *60*, 2115–2128. <https://doi.org/10.1007/s10722-013-9979-y>.
44. Fasuyi, A.O. Nutrient stock and phytic acid in edible wild leafy vegetables of South Western Nigeria. *J. Food Agric. Environ.* **2005**, *3*, 35–39. <https://doi.org/10.3923/pjn.2005.37.42>.
45. Ziena, H.M. Proximate composition and mineral contents of amaranth leaves as affected by varieties and planting dates. *Alexandria J. Food Sci. Technol.* **2000**, *1*, 12–21.
46. Singh, P.; Mishra, S.; Sahoo, S. Nutritional evaluation of dehydrated spinach (*Spinacia oleracea* L.) leaves. *J. Food Sci. Technol.* **2014**, *51*, 123–129.
47. Sarker, U.; Oba, S. Response of nutrients, minerals, antioxidant leaf pigments, vitamins, polyphenol, flavonoid and antioxidant activity in selected vegetable amaranth under four soil water content. *Food Chem.* **2018**, *252*, 72–83. <https://doi.org/10.1016/j.foodchem.2018.01.097>. PMID: 29478565.
48. Berganza, B.E.; Moran, A.W.; Rodríguez, G.M.; Coto, N.M.; Santamaría, M.; Bressani, R. Effect of variety and location on the total fat, fatty acids and squalene content of amaranth. *Plant Foods Hum. Nutr.* **2003**, *58*, 1–6. <https://doi.org/10.1023/B:QUAL.0000041143.24454.0a>.
49. Temesgen, A.; Bultosa, G. Chapter 2.1—Amaranth leaves and seeds nutrients and bioactive compounds potential for nutrition and health. In *Processing, Nutrition and Value Addition Principles of Neglected and Underutilized African Foods*; Workneh, T.S., Mbugue, D.O., Bultosa, G., Eds.; Academic Press: Cambridge, MA, USA, 2026; pp. 75–94. <https://doi.org/10.1016/B978-0-443-33433-7.00006-X>.
50. Fasuyi, A.O.; Dairo, F.A.S.; Adeniji, A.O. Tropical vegetable (*Amaranthus cruentus*) leaf meal as alternative protein supplement in broiler starter diets: Bionutritional evaluation. *J. Cent. Eur. Agric.* **2008**, *9*, 23–34.
51. Bonasia, A.; Lazzizzera, C.; Elia, A.; Conversa, G. Nutritional, Biophysical and Physiological Characteristics of Wild Rocket Genotypes as Affected by Soilless Cultivation System, Salinity Level of Nutrient Solution and Growing Period. *Front. Plant Sci.* **2017**, *8*, 300. <https://doi.org/10.3389/fpls.2017.00300>.
52. Roupheal, Y.; Kyriacou, M.C. Enhancing Quality of Fresh Vegetables Through Salinity Eustress and Biofortification Applications Facilitated by Soilless Cultivation. *Front. Plant Sci.* **2018**, *9*, 1254. <https://doi.org/10.3389/fpls.2018.01254>.
53. Sarker, U.; Hossain, M.N.; Oba, S.; Ercisli, S.; Marc, R.A.; Golokhvast, K.S. Salinity Stress Ameliorates Pigments, Minerals, Polyphenolic Profiles, and Antiradical Capacity in Lalshak. *Antioxidants* **2023**, *12*, 173. <https://doi.org/10.3390/antiox12010173>.
54. Akin-Idowu, P.E.; Odunola, O.A.; Gbadegesin, M.A.; Ademoyegun, O.T.; Aduloju, A.O.; Olagunju, Y.O. Nutritional Evaluation of Five Species of Grain Amaranth—An Underutilized Crop. *Int. J. Sci.* **2017**, *6*, 18–27. <https://doi.org/10.18483/ijSci.1131>.
55. Jiménez-Aguilar, D.M.; Grusak, M.A. Minerals, Vitamin C, Phenolics, Flavonoids and Antioxidant Activity of *Amaranthus* Leafy Vegetables. *J. Food Compos. Anal.* **2017**, *58*, 33–39. <https://doi.org/10.1016/j.jfca.2017.01.005>.
56. Manyelo, T.G.; Sebola, N.A.; Hassan, Z.M.; Mabelebele, M. Characterization of the Phenolic Compounds in Different Plant Parts of *Amaranthus cruentus* Grown under Cultivated Conditions. *Molecules* **2020**, *25*, 4273. <https://doi.org/10.3390/molecules25184273>.
57. Rastogi, A.; Shukla, S. Amaranth: A New Millennium Crop of Nutraceutical Values. *Crit. Rev. Food Sci. Nutr.* **2013**, *53*, 109–125. <https://doi.org/10.1080/10408398.2010.517876>.
58. Iyaka, Y.A.; Idris, S.; Alawode, R.A.; Bagudo, B.U. Nutrient content of selected edible leafy vegetables. *Am. J. Appl. Chem.* **2014**, *2*, 42–45. <https://doi.org/10.11648/j.ajac.20140203.12>.
59. Nwaogu, L.A.; Ujowundu, C.O.; Mgbemena, A. Studies on the nutritional and phytochemical composition of *Amaranthus hybridus* leaves. *Bio-Res.* **2006**, *4*, 28–31. <https://doi.org/10.4314/br.v4i1.28608>.
60. Yiblet, Y.; Adamu, E. Nutritional composition and phytochemical evaluation of some selected wild edible plants in tach gaint district, northwestern Ethiopia. *Sci. World J.* **2023**, *1*, 6670648. <https://doi.org/10.1155/2023/6670648>.
61. Sarker, U.; Oba, S.; Daramy, M.A. Nutrients, minerals, antioxidant pigments and phytochemicals, and antioxidant capacity of the leaves of stem amaranth. *Sci. Rep.* **2020**, *10*, 3892. <https://doi.org/10.1038/s41598-020-60252-7>.
62. Sarker, U.; Oba, S. Drought stress enhances nutritional and bioactive compounds, phenolic acids and antioxidant capacity of *Amaranthus* leafy vegetable. *BMC Plant Biol.* **2018**, *18*, 258. <https://doi.org/10.1186/s12870-018-1484-1>.
63. Goddek, S.; Delaide, B.; Mankasingh, U.; Ragnarsdottir, K.V.; Jijakli, M.H.; Thorarinsdottir, R. Challenges of sustainable and commercial aquaponics. *Sustainability* **2015**, *7*, 4199–4224. <https://doi.org/10.3390/su7044199>.
64. Ondo, J.A.; Biyogo, R.M.; Mebale, A.J.A.; Eba, F. Uptake of copper, zinc and other metals by *Amaranthus cruentus* irrigated with contaminated effluent. *J. Environ. Chem. Eng.* **2015**, *3*, 2875–2881. <https://doi.org/10.1016/j.jece.2015.10.022>.

65. Tózsér, D.; Yelamanova, A.; Sipos, B.; Magura, T.; Simon, E. A meta-analysis on the heavy metal uptake in *Amaranthus* species. *Environ. Sci. Pollut. Res.* **2023**, *30*, 85102–85112. <https://doi.org/10.1007/s11356-023-28374-3>.
66. Li, H.; Deng, Z.; Liu, R.; Zhu, H.; Draves, J.; Marcone, M.; Sun, Y.; Tsao, R. Characterization of phenolics, betacyanins and antioxidant activities of the seed, leaf, sprout, flower and stalk extracts of three *Amaranthus* species. *J. Food Compos. Anal.* **2015**, *37*, 57–68. <https://doi.org/10.1016/j.jfca.2014.09.003>.
67. Escalante-Aburto, A.; Malfait-Juárez, I.; Ponce-García, N.; Mariscal-Moreno, R.M. Chapter 7—Amaranth as a functional food and ingredient for the food industry. In *Improving Health and Nutrition Through Functional Foods*; Campos, M.R.S., Ed.; Woodhead Publishing: Cambridge, UK, 2025; pp. 127–141. <https://doi.org/10.1016/B978-0-443-21874-3.00011-8>.
68. Araujo-León, J.A.; Sánchez-del Pino, I.; Ortiz-Andrade, R.; Hidalgo-Figueroa, S.; Carrera-Lanestosa, A.; Brito-Argáez, L.G.; González-Sánchez, A.; Giacomán-Vallejos, G.; Hernández-Abreu, O.; Peraza-Sánchez, S.R.; et al. HPLC-Based Metabolomic Analysis and Characterization of *Amaranthus cruentus* Leaf and Inflorescence Extracts for Their Antidiabetic and Antihypertensive Potential. *Molecules* **2024**, *29*, 2003. <https://doi.org/10.3390/molecules29092003>.
69. Miguel, M.G. Betalains in Some Species of the Amaranthaceae Family: A Review. *Antioxidants* **2018**, *7*, 53. <https://doi.org/10.3390/antiox7040053>.
70. Howard, J.E.; Villamil, M.B.; Riggins, C.W. Amaranth as a natural food colorant source: Survey of germplasm and optimization of extraction methods for betalain pigments. *Front. Plant Sci.* **2022**, *13*, 932440. <https://doi.org/10.3389/fpls.2022.932440>.
71. Roriz, C.L.F. New Natural Sources of Betacyanins: Optimization of the Extraction Process, Stability Studies, and Incorporation as a Colorant in Different Foods. Ph.D. Thesis, Universidad Complutense de Madrid, Madrid, Spain, 13 July 2021.
72. Sarker, U.; Rabbani, M.G.; Oba, S.; Eldehna, W.M.; Al-Rashood, S.T.; Mostafa, N.M.; Eldahshan, O.A. Phytonutrients, Colorant Pigments, Phytochemicals, and Antioxidant Potential of Orphan Leafy *Amaranthus* Species. *Molecules* **2022**, *27*, 2899. <https://doi.org/10.3390/molecules27092899>.
73. Martinez, R.M.; Melo, C.P.B.; Pinto, I.C.; Mendes-Pierotti, S.; Vignoli, J.A.; Verri, W.A.; Casagrande, R. Betalains: A Narrative Review on Pharmacological Mechanisms Supporting the Nutraceutical Potential Towards Health Benefits. *Foods* **2024**, *13*, 3909. <https://doi.org/10.3390/foods13233909>.
74. Ștefănescu, C.; Voștinariu, O.; Mogoșan, C.; Crișan, G.; Balica, G. The Neuroprotective Potential of Betalains: A Focused Review. *Plants* **2025**, *14*, 994. <https://doi.org/10.3390/plants14070994>.
75. Li, G.; Meng, X.; Zhu, M.; Li, Z. Research Progress of Betalain in Response to Adverse Stresses and Evolutionary Relationship Compared with Anthocyanin. *Molecules* **2019**, *24*, 3078. <https://doi.org/10.3390/molecules24173078>.
76. Pompelli, M.F.; Arrieta, D.V.; Rodríguez, Y.Y.P.; Ramírez, A.M.J.; Bettin, A.M.V.; Avilez, M.A.Q.; Cárcamo, J.A.A.; García-Castaño, S.G.; González, L.M.M.; Cordero, E.D.F.; et al. Can Chlorophyll a Fluorescence and Photobleaching Be a Stress Signal under Abiotic Stress in *Vigna unguiculata* L.? *Sustainability* **2022**, *14*, 15503. <https://doi.org/10.3390/su142315503>.
77. Sarker, U.; Oba, S.; Ercisli, S.; Assouguem, A.; Alotaibi, A.; Ullah, R. Bioactive Phytochemicals and Quenching Activity of Radicals in Selected Drought-Resistant *Amaranthus tricolor* Vegetable Amaranth. *Antioxidants* **2022**, *11*, 578. <https://doi.org/10.3390/antiox11030578>.
78. Prior, R.L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290–4302.
79. Park, S.J.; Sharma, A.; Lee, H.J. A Review of Recent Studies on the Antioxidant Activities of a Third-Millennium Food: *Amaranthus* spp. *Antioxidants* **2020**, *9*, 1236. <https://doi.org/10.3390/antiox9121236>.
80. Nana, F.W.; Hilou, A.; Millogo, J.F.; Nacoulma, O.G. Phytochemical Composition, Antioxidant and Xanthine Oxidase Inhibitory Activities of *Amaranthus cruentus* L. and *Amaranthus hybridus* L. Extracts. *Pharmaceuticals* **2012**, *5*, 613–628. <https://doi.org/10.3390/ph5060613>.
81. Jimoh, M.O.; Afolayan, A.J.; Lewu, F.B. Antioxidant and phytochemical activities of *Amaranthus caudatus* L. harvested from different soils at various growth stages. *Sci. Rep.* **2019**, *9*, 12965. <https://doi.org/10.1038/s41598-019-49276-w>.
82. Karamać, M.; Gai, F.; Longato, E.; Meineri, G.; Janiak, M.A.; Amarowicz, R.; Peiretti, P.G. Antioxidant Activity and Phenolic Composition of Amaranth (*Amaranthus caudatus*) during Plant Growth. *Antioxidants* **2019**, *8*, 173. <https://doi.org/10.3390/antiox8060173>.
83. Bastos, E.L.; Schliemann, W. Betalains as Antioxidants. In *Plant Antioxidants and Health*; Reference Series in Phytochemistry; Ekiert, H.M., Ramawat, K.G., Arora, J., Eds.; Springer: Cham, Switzerland, 2021. https://doi.org/10.1007/978-3-030-45299-5_9-2.
84. Gandía-Herrero, F.; Escribano, J.; García-Carmona, F. Purification and antiradical properties of the structural unit of betalains. *J. Nat. Prod.* **2012**, *75*, 1030–1036. <https://doi.org/10.1021/np200950n>.

85. Sadowska-Bartosz, I.; Bartosz, G. Biological Properties and Applications of Betalains. *Molecules* **2021**, *26*, 2520. <https://doi.org/10.3390/molecules26092520>.
86. Khan, M.I. Plant betalains: Safety, antioxidant activity, clinical efficacy, and bioavailability. *Compr. Rev. Food Sci. Food Saf.* **2016**, *15*, 316–330. <https://doi.org/10.1111/1541-4337.12185>.
87. Paško, P.A.W.E.Ł.; Bartoń, H.; Fołta, M.A.R.I.A.; Gwizdź, J. Evaluation of antioxidant activity of amaranth (*Amaranthus cruentus*) grain and by-products (flour, popping, cereal). *Rocz. Panstw. Zakl. Hig.* **2007**, *58*, 35–40. PMID: 17711088.
88. Nishanth, D.; Alshamsi, M.H.; Alkaabi, A.M.; AlKaabi, A.H.; Alnuaimi, S.H.; Nair, C.S.; Jaleel, A. Aquaponics as a climate-smart technology for sustainable food production: A comparison with conventional production system in United Arab Emirates. *J. World Aquac. Soc.* **2024**, *55*, e13049. <https://doi.org/10.1111/jwas.13049>.
89. Albadwawi, M.A.O.K.; Ahmed, Z.F.R.; Kurup, S.S.; Alyafei, M.A.; Jaleel, A. A Comparative Evaluation of Aquaponic and Soil Systems on Yield and Antioxidant Levels in Basil, an Important Food Plant in Lamiaceae. *Agronomy* **2022**, *12*, 3007. <https://doi.org/10.3390/agronomy12123007>.
90. Gudžinskaitė, I.; Laužikė, K.; Pukalskas, A.; Samuoliene, G. Light Modulation of Photosynthate Accumulation in Microgreens Grown in a Controlled Environment During Storage. *Horticulturae* **2025**, *11*, 176. <https://doi.org/10.3390/horticulturae11020176>.
91. Netshimbupfe, M.H.; Berner, J.; Van Der Kooy, F.; Oladimeji, O.; Gouws, C. Influence of Drought and Heat Stress on Mineral Content, Antioxidant Activity and Bioactive Compound Accumulation in Four African *Amaranthus* Species. *Plants* **2023**, *12*, 953. <https://doi.org/10.3390/plants12040953>.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.