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Growth and Quality of Leaf and Romaine Lettuce Grown on a Vertical Farm in an Aquaponics System: Results of Farm Research

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Abstract: The integration of indoor vertical cultivation with a recirculating aquaculture system into an aquaponic system has the potential to become one of the most effective sustainable production systems for fish and leafy vegetables. In this study, lettuce was produced on rafts in a coupled recirculation aquaponic system in the plant factory under controlled environmental conditions. The aims of this study were to evaluate the yield, mineral status, and health-promoting bioactive compounds of leaf and romaine lettuce cultivars grown in a recirculating aquaponic system. The yield and biometric parameters and quality parameters of lettuce leaves (nitrate, mineral, L-ascorbic acid, carotenoid, phenolic compound, and total polyphenolic contents) were examined. Monitoring of the water in the aquaponic system showed a low concentration of nitrates, phosphorus (P), potassium (K), and magnesium (Mg), but the proportion of mineral nutrients as well as pH were stable throughout the lettuce cultivation period. The heads of romaine lettuce 'Yakina', 'Pivotal', and 'Waygo' reached a fresh weight of 86 g, on average, 23% higher than the leaf lettuce 'Nordice' over a three-week cultivation period. Despite the low nutrient concentration in the aquaponic solution, the nutrient status of the romaine lettuces 'Yakina' and 'Pivotal' was within the optimal range. The concentrations of chlorophyll *a* and carotenoids in 'Yakina' and 'Pivotal' were higher than those in 'Nordice' and 'Waygo'. The nitrate, phosphorus, and potassium contents in the leaves of 'Nordice' and 'Waygo' were below the optimal range; however, their polyphenol concentrations were the highest. Our results indicate that the effectiveness of aquaponic cultivation of lettuce in terms of biomass production and the nutritional and health-promoting value of lettuce depends on the plant genotype.

Keywords: aquaponics; lettuce; plant factories with artificial lighting; plant nutrition; phytochemicals; sustainable agriculture

Citation: Matysiak, B.; Kaniszewski, S.; Mieszczakowska-Frąc, M. Growth and Quality of Leaf and Romaine Lettuce Grown on a Vertical Farm in an Aquaponics System: Results of Farm Research. *Agriculture* **2023**, *13*, 897. https://doi.org/10.3390/agriculture13040897

Academic Editors: Sanja Fabek Uher, Marko Petek, Goran Fruk and Ronald K. Luz

Received: 6 March 2023
Revised: 11 April 2023
Accepted: 17 April 2023
Published: 19 April 2023

1. Introduction

Plant factories with artificial lighting were developed over 20 years ago and are an important solution to many environmental problems and food security using a closed environment and its control [1]. This technology, especially vertical farming, is of interest around the world due to its high and stable productivity, high resource use efficiency, and high environmental benefits [2]. Plant factories can be located in cities or suburban areas, which significantly shortens the food supply chain from the producer to the consumer. Soilless hydroponic systems are used in all plant factories to enable constant production of vegetables all year around in a controlled environment. Despite the many advantages of vertical farming, the carbon footprint of this technology is 5.6–16.7 times greater than that of other commonly used methods [3]. Therefore, new technologies enabling a sustainable vertical farming industry should be developed [4]. Monsees et al. [5] showed that the production of lettuce in separate aquaponic systems achieves the same yield and quality as conventional hydroponic systems, but greenhouse gas emissions are drastically reduced due to inorganic fertilizer savings. The decoupled aquaponic technology proposed by Monsees et al. [5] can be optimized by using a coupled system in which environmental conditions for fish, microbes, and plants, such as pH, EC, and water temperature, are optimized. In addition, it would be possible to use organic fertilizers in hydroponics as an alternative to minerals, which is crucial to meeting the challenges of agriculture and climate change around the world.

Aquaponics is a food production system that combines recirculating aquaculture and hydroponics, where nutrient-rich aquaculture water is supplied to hydroponically grown plants [6,7]. This system has gained global attention in recent years because of its high water- and nutrient-use efficiency. In addition, it does not require the discharge of organic effluents as pollutants. Waste material excreted from fish is mineralized via biochemical conversions to release essential nutrients for plants and is used as a liquid fertilizer for the hydroponic production of vegetables. The current state of knowledge highlights the possibility of using aquaculture water to hydroponically produce lettuce in open field and greenhouse conditions [5,8]. Only a few reports concern the use of aquaponics in plant factories, including the use of salt water [9]. The challenge for science and practice remains the development of efficiently functioning aquaponics in plant factories for various species of vegetables based on the rearing of various fish species.

Lettuce (*Lactuca sativa* L.) is an economically important vegetable and a significant source of essential minerals, as well as vitamins and naturally health-promoting phytochemicals, including flavonoids, carotenoids, and L-ascorbic acid [10,11]. The mineral composition and content of bioactive compounds vary between horticultural types of lettuce [12] and depend on environmental factors and cultivation systems. Among the looseleaf, butterhead, crisphead, and romaine types of lettuce, romaine lettuce is the richest source of β-carotene (4 mg 100 g^{-1} FW). Loose-leaf lettuce has the highest total phenolic compounds (338 mg 100 g^{-1}) and is a moderate source of vitamin C and β-carotene. The accumulation of minerals and bioactive compounds in lettuce strongly depends on environmental factors, such as temperature [13] and the quality, intensity, and duration of light [14–17]. Previous studies have also indicated that nitrogen deficiency favorably affects the biosynthesis of phenolic acids, flavonols, anthocyanins, and ascorbic acid in lettuce [18,19].

The aim of the current research was to check the possibility of obtaining an adequate yield and good quality of leaf and romaine lettuce grown in a recirculating aquaponic system using only organic fertilizers produced by fish without supplementing with mineral fertilizers in a plant factory and to determine the mineral status and health-promoting bioactive compound content in lettuce leaves. The main goal was to implement our previous research results, conducted on a laboratory scale, in a plant factory operating on a full commercial scale.

2. Materials and Methods

2.1. Plants and Fish Used in this Study

Four cultivars of lettuce were used in the study: one cultivar of leaf lettuce (*Lactuca sativa* L.) named 'Nordice', three cultivars of romaine lettuce (*Lactuca sativa* var. *longifolia* Lam.), two midi-types ('Pivotal' and 'Yakina'), and one mini-type ('Waygo'). The lettuce seedlings were produced in mineral wool (Grodan) and planted on September 19, 2022. Hybrids of Siberian sturgeon (*Acipenser baerii*, Brandt) and Russian sturgeon (*Acipenser gueldensaedtii*, Brandt, and Ratzeburg) were used in aquaculture due to high values of performance, breeding, and rearing, and because this hybrid is more economically efficient than the rearing of purebred species [20].

2.2. Aquaculture

The lettuce was grown on a vertical farm in a recirculating aquaculture system (Plantlab, Kaszewy Kościelne, Poland). The system was operated with fish and plants for three months prior to the start of the study to increase nutrient levels and ensure sufficient plant growth. Vertical lettuce cultivation and fish rearing tanks were in separate halls.

Three-week-old seedlings were planted on specially designed floating polystyrene rafts (0.9 m × 0.6 m) with holes (24 pieces on the raft) for rockwool cubes (Grodan) at a planting density of 40 seedlings per square meter. Plants were grown in hydroponic beds (40.0 m × 1.8 m) arranged on eleven levels, and two hydroponic beds were intended for the experimental study (Figure 1). The depth of water in the hydroponic beds was 15 cm. The multi-level construction for plant cultivation was located in a production hall with a fully controlled atmosphere and no access to sunlight. The temperature was maintained at 23 °C, and the humidity was at 75% RH. The source of light for plants was specially designed LED lamps (70:18:12; Red:Green:Blue), based on the results of our previous study of romaine lettuce [21]. PPFD was 200–220 µmol m^2 s^{-1}, and the photoperiod was 15 h.

(**a**) (**b**)

Figure 1. Experimental lettuce cultivation facility on a vertical farm, (**a**) lettuce grown in hydroponic beds, (**b**) raft with visible root system of romaine lettuce.

The fish were fed in accordance with the recommendations of the Institute of Ichthyobiology and Aquaculture of the Polish Academy of Sciences (Gołysz, Poland). Fish feed contained 7.63% N and other ingredients, such as 7950 mg/kg DW P, 28,600 mg/kg DW K, and 9700 mg/kg DW. Ca, 2820 mg/kg DW Mg, 466 mg/kg DW Fe, 42.2 mg/kg DW Mn, 21 mg/kg DW Cu, 130 mg/kg DW Zn, and 28.2 mg/kg DW B. The chemical composition of the tap water used to fill tanks for fish rearing was as follows: pH 7.2, EC 0.73 mS/cm, hardness 23.8°dH and ingredients such as 332 mg/L HCO_3^-, 1.04 mg/L N-NO_3^-, <0.05 mg/L N-NH_4^+, 0.07 mg/L P-PO_4^{3-}, 2.98 mg/L K^+, 132 mg/L Ca^{2+}, 22.8 mg/L Mg^{2+}, 15.5 mg/L Na^+, 44.2 mg/L Cl^-, 108 mg/L SO_4^{2-}, 0.01 mg/L Fe, 0.01 mg/L Mn, <0.02 mg/L Cu, 5.13 mg/L Zn, and 0.04 mg/L B. One tank for fish rearing with a capacity of 60 m^3 was connected to two experimental hydroponic beds for lettuce growth. The stocking density of fish was 6.5 kg m^{-3}. Water from the fish tanks was pumped to a reactor with beneficial microorganisms to oxidize NH_4^+ and then, after filtering out the solids, to a cultivation hall where lettuce was grown on floating rafts.

2.3. Mineral Composition of Water in an Aquaponic System

Water for analysis was taken from the hydroponic beds four times at weekly intervals. The electrical conductivity (EC), pH, and mineral nutrient content of the water were determined. The pH and N-NO_3 contents were analyzed by the potentiometric method using the Thermo Scientific apparatus model Orion Versastar (Beverly, MA, USA), and the EC by the conductivity method. The concentrations of P, K, Ca, Mg, and SO_4 were determined following the spectrophotometric method using an inductively coupled plasma sequential emission spectrometer (ICP Perkin-Elmer model Optima 2000 DV, Boston, MA, USA).

2.4. Yield and Biometric Parameters

Lettuce was harvested when it reached commercial maturity, i.e., 21 days after transplant, after which the fresh and dry weight of leaves, number of leaves, head circumference (for romaine lettuces forming heads), plant height and diameter, fresh weight, and root length were measured. A visual quality score on a scale of 1 to 5, where 1 means the worst and 5 means the best plant, was also assessed. Measurements of biometric parameters were performed on 18 randomly selected plants collected from different places in hydroponic beds.

2.5. Chlorophyll, Flavonol, and Nitrogen Balance Indices

The chlorophyll (Cl), nitrogen (nitrogen balance index), and flavonol (FLAV) indices were measured using the Dualex Scientific+ (Force A, Orsay, France) lefe-clip device. Measurements were made on 36 fully expanded leaves from each cultivar.

2.6. Macro- and Micronutrients in Plants

Nitrates in fresh plant material were extracted with water at a ratio of 1:10 and determined by the potentiometric method using the Thermo Scientific apparatus model Orion Versastar (Beverly, MA, USA). The content of the other components was determined by the dried plant material. The contents of P, K, Ca, Mg, Fe, Mn, Cu, Zn, and B were determined in three replications using an ICP spectrometer. The N content was analyzed using the Kjeldahl method (Vapodest Kjeldahl apparatus, Gerhardt GmbH & Co., KG, Königswinter, Bonn, Germany) [22]. Selected elements were determined at their characteristic wavelengths [23]. All nutrients were determined in three replications.

2.7. Phytochemical Content

2.7.1. Chlorophyll and Carotenoids

The contents of carotenoid and chlorophyll were measured using a spectrophotometric method, as maintained by Lichtenthaler and Wellburn [24]. The lettuce samples, after disintegration, were homogenized with a cold solution of 80% acetone. The extracts were centrifuged at 4 °C for 15 min at 10 000 rpm. Then, 0.5 mL of supernatant was added to 4.5 mL of acetone solution (80%). The carotenoid and chlorophyll contents were determined using a UV-VIS spectrophotometer (Cary 300 Bio), based on extract absorbance at three wavelengths: 470 nm—carotenoids; 645 nm—chlorophyll *a*; and 663 nm—chlorophyll *b*. Total chlorophyll was calculated as the sum of chlorophyll *a* and chlorophyll *b*. The results were expressed in mg 100 g^{-1} of fresh sample weight (FW).

2.7.2. L-Ascorbic, Citric, and Malic Acid

The content of L-ascorbic acid was determined following the HPLC method (high performance liquid chromatography) using an Agilent Technologies HPLC system (1200 series) equipped with a diode array detector (DAD). The separation was applied using a Supelcosil LC-18 column (250 mm × 4.6 mm; 5 µm) with a pre-column and a solution of phosphate-buffered (1% KH_2PO_4; 2.5 pH) as the mobile phase. The column was maintained at a temperature of 30 °C, and the mobile phase flow rate was 0.8 mL/min. The detection of ascorbic acid was performed by 244 nm absorbance. Samples before HPLC injection were dissolved in 6% HPO_3. The results were expressed as mg 100 g^{-1} FW.

2.7.3. Flavonoids

The content of the flavonoids in lettuce was measured using a spectrophotometric method [25,26]. The lettuce leaves were homogenized with a cold solution of acetone (80%)

and filtered using reduced pressure. Then, acetone was removed from the supernatant by evaporating at 45 °C. The aqueous solution was subjected to centrifugation at 8000 rpm for 10 min. Then, 0.25 mL of the extract was mixed with 0.075 mL of $NaNO_2$ solution (5%) and 1.25 mL of distilled water and kept for 6 min. Subsequently, 10% $AlCl_3$ solution was added (0.15 mL) and kept for 5 min. Next, 0.5 mL of a solution of 1 M NaOH was added. The total mixture was mixed thoroughly. Samples were made up of distilled water in a 2.5 mL volume, and the measurement was applied by absorbance at 510 nm on a spectrophotometer (UviLine 9400) against a blank. The flavonoid content was expressed in mg 100 g^{-1} FW.

2.7.4. Total Polyphenolic Content (TPC)

The total polyphenol content (TPC) was measured using a modified spectrophotometric method [27]. The plant sample was homogenized with 70% ethanol. The homogenate was centrifuged for 10 min at 20,000× g, following the mixture of 0.4 mL supernatant with 1.6 mL sodium carbonate solution (7.5%). Then, 2 mL of Folin–Ciocalteu phenol reagent was added, and the mixture was shaken. After incubation for 30 min at ambient temperature in the dark, the absorbance was read against the prepared blank at 765 nm. The polyphenol content was expressed as mg of gallic acid equivalents in mg 100 g^{-1} FW.

2.8. Statistical Analysis

A one-way analysis of variance (ANOVA) was used to test statistical differences between the means using SAS software version 9.1 (StatSoft Inc., Tulsa, OK, USA). Tukey's HSD test was applied at $p \leq 0.05$ for the mean comparison of the tested cultivars.

3. Results

3.1. Mineral Composition of Water in an Aquaponic System

Monitoring of water in an aquaponic system showed a good balance of mineral nutrients, as the $N\text{-}NO_3^-$, $N\text{-}NH_4^+$, P, K, Ca, and Mg contents remained at the same level throughout the lettuce cultivation period (Table 1). No excessive depletion or accumulation of macronutrients was demonstrated. The average contents of macronutrients are: 46 mg/L $N\text{-}NO_3^-$, 3 mg/L $N\text{-}NH_4^+$, 3 mg/L P, 19 mg/L K, 141 mg/L Ca, and 27 mg/L Mg. Similarly, the pH (7.3) and EC (0.89 mS/cm) of the water remained at similar levels.

Table 1. The electrical conductivity EC (mS/cm), pH, and mineral composition ($N\text{-}NO_3^-$, $N\text{-}NH_4^+$, P, K, Ca, and Mg) of aquaponic nutrient solution during lettuce cultivation.

Assessment Date	EC (mS/cm)	pH	$N\text{-}NO_3^-$ mg/L	$N\text{-}NH_4^+$ (mg/L)	P (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)
19 September	0.90 a	7.40 a	45.9 a	2.8 a	2.60 a	20.0 a	142.0 a	27.6 a
26 September	0.87 a	7.35 a	46.0 a	2.3 a	3.67 a	18.2 a	136.5 a	26.1 a
3 October	0.87 a	7.20 a	47.6 a	4.1 a	3.28 a	17.6 a	142.0 a	27.0 a
11 October	0.93 a	7.25 a	44.4 a	2.8 a	2.70 a	19.0 a	143.0 a	27.5 a

Means followed by the same letter are not significantly different ($p < 0.05$) using Tukey's HSD test.

3.2. Yield and Biometric Parameters

The examined morphological characteristics of the lettuce (Figure 1) showed that the yield of leaf lettuce 'Nordice' was significantly lower than the yield of the romaine lettuce cultivars after three weeks of growth. 'Nordice' reached a fresh weight of 70 g and formed 17 leaves. These plants were the shortest (the height was 13 cm) but had the largest diameter, although they were not significantly taller than 'Pivotal' plants. 'Nordice' produced the lowest fresh root weight, but the roots were the longest. The heads of the romaine lettuce reached a fresh weight of 86 g (average for three cultivars), 23% more than the leaf lettuce 'Nordice'. Although the romaine lettuce cultivars reached the same fresh weight, they differed significantly in terms of morphological traits. The 'Waygo' cultivar was the most compact among the evaluated romaine lettuce cultivars. These plants were the shortest and had the lowest diameter; however, they had the highest head circumference and produced the most leaves. The dry matter of lettuce for all tested cultivars under aquaponic conditions was 3.9%. Overall, all lettuce cultivars grown under an aquaponic system received a high visual quality rating, although the score for 'Waygo' was slightly lower due to the occurrence of tip burn symptoms.

3.3. Chlorophyll, Flavonol, and Nitrogen Balance Indices

The leaf chlorophyll index (CI) and nitrogen balance index (NBI) for all tested lettuce cultivars were closely related (Figure 2). 'Pivotal' and 'Waygo' had the highest CI and NBI, and 'Nordice' had the lowest values for these indices. Both CI and NBI for 'Pivotal' and 'Waygo' were as much as five times higher than those for 'Nordice'. The flavonol index for the tested cultivars did not differ much; however, this index for 'Waygo' was significantly higher than that for 'Yakina'.

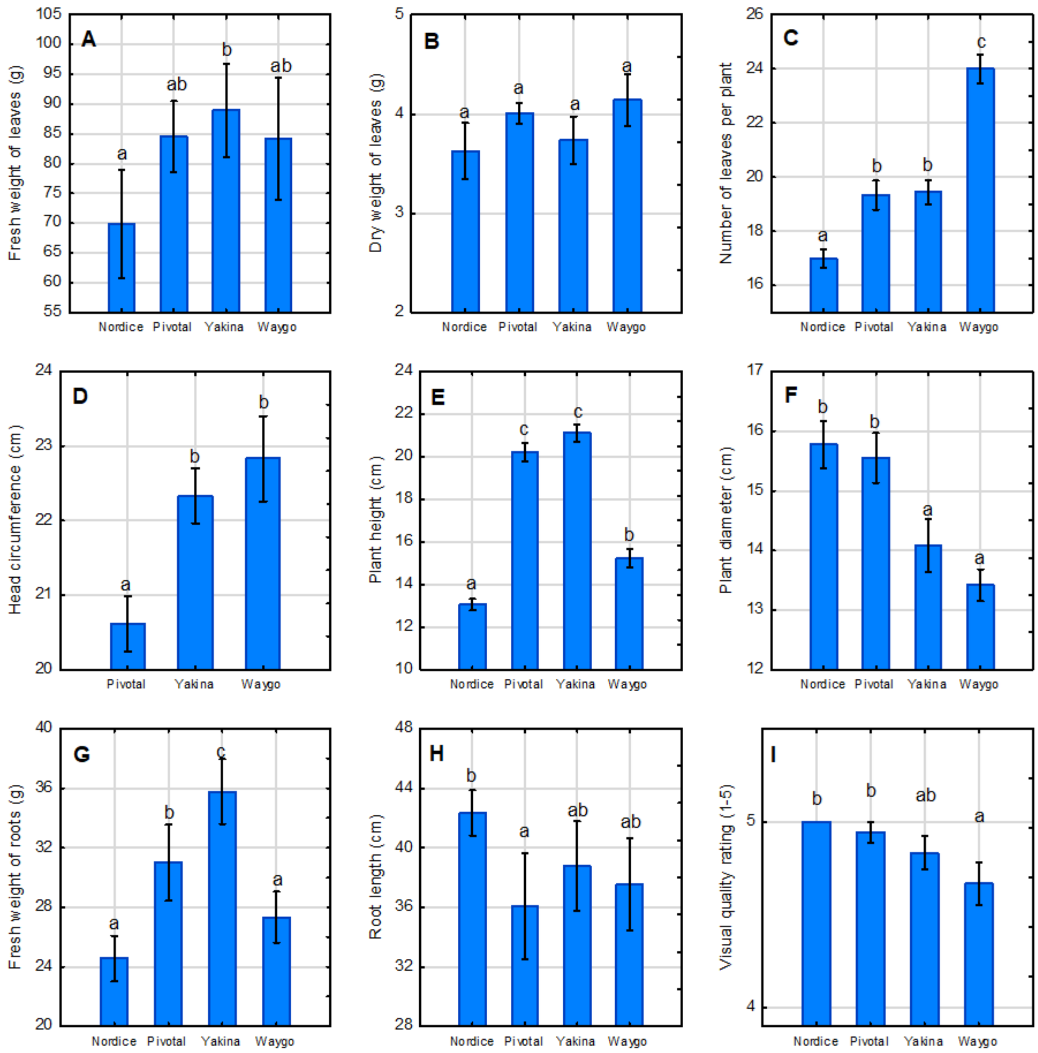

Figure 2. Fresh (**A**) and dry weight of leaves (**B**), number of leaves per plant (**C**), head circumference (**D**), plant height (**E**), plant diameter (**F**), fresh root weight (**G**), root length (**H**), and visual quality scores (**I**) of three romaine lettuce cultivars ('Pivotal', 'Yakina', and 'Waygo') and one leaf lettuce cultivar ('Nordice') grown on a vertical farm in an aquaponic system. Vertical bars represent the standard errors of the means. Different letters indicate statistically significant differences between cultivars at the $p < 0.05$ level according to Tukey's HSD test.

3.4. Macro- and Micronutrients in Plants

Plant nutrient accumulation differed significantly among the cultivars (Tables 2 and 3). The loose-leaf cultivar 'Nordice' had the lowest nitrate and total N, P, K, and Mg contents, as well as low concentrations of Fe, Mn, Cu, and Zn; however, it had the highest Ca content. No significant difference was found between the romaine lettuce cultivars in terms of nitrate (1962 mg kg^{-1} f.w.), total N (3.24%), Mg (1962 mg kg^{-1} d.w.), and Fe (93.91 mg kg^{-1} d.w.) contents (average values). The 'Pivotal' and 'Yakina' cultivars were characterized by very similar P (5325 mg kg^{-1} d.w.), K (73,733 mg kg^{-1} d.w.), and Ca (19,500 mg kg^{-1} d.w.) contents, and these values were significantly higher than those determined for the 'Waygo' cultivar.

Table 2. Concentrations of nitrates (mg kg^{-1} FW), total nitrogen (%), and macroelements P, K, Ca, and Mg (in mg kg^{-1} DW) in the leaves of one leaf lettuce cultivar ('Nordice') and three romaine lettuce cultivars ('Pivotal', 'Yakina', and 'Waygo') grown on a vertical farm in an aquaponic system.

Cultivar	NO_3 (mg kg^{-1} FW)	N (%)	P (mg kg^{-1} DW)	K (mg kg^{-1} DW)	Ca (mg kg^{-1} DW)	Mg (mg kg^{-1} DW)
Nordice	885 ± 142 a	2.33 ± 0.10 a	2557 ± 203 a	27033 ± 2001 a	27133 ± 1241 c	885 ± 142 a
Pivotal	1967 ± 126 b	3.11 ± 0.11 b	5130 ± 179 c	73000 ± 2409 c	20500 ± 1021 b	1967 ± 126 b
Yakina	2221 ± 357 b	3.45 ± 0.04 b	5520 ± 246 c	74467 ± 2577 c	18500 ± 265 b	2221 ± 357 b
Waygo	1699 ± 221 ab	3.17 ± 0.11 b	4100 ± 240 b	40167 ± 2134 b	14233 ± 338 a	1699 ± 221 ab

Different letters indicate statistically significant differences between cultivars at the $p < 0.05$ level according to Tukey's HSD test.

Table 3. Concentrations of microelements (Fe, Mn, Cu, Zn, and B, in mg kg^{-1} DW) in the leaves of leaf lettuce 'Nordice' and romaine lettuce 'Pivotal', 'Yakina', and 'Waygo' grown on a vertical farm in an aquaponic system.

Cultivar	Fe (mg kg^{-1} DW)	Mn (mg kg^{-1} DW)	Cu (mg kg^{-1} DW)	Zn (mg kg^{-1} DW)	B (mg kg^{-1} DW)
Nordice	75.97 ± 4.49 a	23.27 ± 1.51 a	2.81 ± 0.24 a	110.33 ± 2.73 a	23.9 ± 1.27 a
Pivotal	93.87 ± 5.74 ab	131.00 ± 12.22 b	5.56 ± 0.11 c	222.33 ± 20.00 b	24.43 ± 0.59 a
Yakina	98.57 ± 0.69 b	115.27 ± 13.8 b	6.78 ± 0.13 d	343.33 ± 28.38 c	24.43 ± 0.27 a
Waygo	89.30 ± 3.89 ab	22.03 ± 0.61 a	3.74 ± 0.23 b	124.00 ± 4.16 a	26.63 ± 0.37 a

Different letters indicate statistically significant differences between cultivars at the $p < 0.05$ level according to Tukey's HSD test.

3.5. Phytochemical Content

The concentration of chlorophyll *a* (Table 4) was higher for 'Pivotal' and 'Waygo' (25.23 mg 100 g^{-1}, on average) than for 'Nordice' and 'Yakina' (19.33 mg 100 g^{-1}, on average), while the content of chlorophyll *b* was similar among the compared cultivars (7.33 mg 100 g^{-1}, on average). Cultivars with the highest chlorophyll *a* content also had the highest chlorophyll *a+b* content. Large variation was observed among the analysed bioactive compounds exhibiting pro-health properties (Tables 4 and 5): carotenoids (4.8–6.9 mg 100 g^{-1}), total polyphenols (30.5–50.4 mg 100 g^{-1}), and L-ascorbic acid (1.09–5.46 mg 100 g^{-1}). The flavonoid content in the leaves of the compared cultivars was similar (41.40 mg 100 g^{-1}, on average). The highest carotenoid content was found in the leaves of 'Pivotal' and 'Waygo' (6.79 and 6.87 mg 100 g^{-1}, respectively). The highest amount of polyphenols (TPC) was found in the leaves of 'Nordice' and 'Waygo' (50.41 and 43.07 mg 100 g^{-1}, respectively). The L-ascorbic acid content in leaves of 'Nordice', 'Pivotal', and 'Waygo' was significantly higher (5.24 mg 100 g^{-1}, on average) than that in leaves of 'Yakina' (1.09 mg 100 g^{-1}). The dominant acid in lettuce leaves was malic acid. The highest total acid content (501 mg 100 g^{-1}), as well as malic acid (445 mg 100 g^{-1}), was found in 'Pivotal', and the lowest content was found in 'Waygo' (301 and 255 mg 100 g^{-1}, respectively). The citric acid content in the leaves of the compared cultivars was similar (45.38 mg 100 g^{-1}, on average).

Table 4. The content of chlorophylls *a* and *b*, carotenoids, flavonoids, and total polyphenols (TPC) in leaf lettuce 'Nordice' and romaine lettuce 'Pivotal', 'Yakina', and 'Waygo' grown on a vertical farm in an aquaponic system.

Cultivar	Chlorophyll *a* (mg 100 g^{-1} FW)	Chlorophyll *b* (mg 100 g^{-1} FW)	Chlorophyll *a+b* (mg 100 g^{-1} FW)	Carotenoids (mg 100 g^{-1} FW)	Flavonoids (mg 100 g^{-1} FW)	TPC (mg 100 g^{-1} FW)
Nordice	20.02 ± 0.27 a	6,63 ± 0.06 a	26.64 ± 0.36 a	4.87 ± 0.11 a	52.56 ± 9.92 a	50.41 ± 2.07 b
Pivotal	25.75 ± 0.80 b	9.41 ± 0.34 a	35.15 ± 1.14 b	6.79 ± 0.24 b	41.44 ± 7.96 a	31.58 ± 2.39 a
Yakina	18.64 ± 0.26 a	5,62 ± 1.08 a	24.26 ± 0.85 a	4.81 ± 0.06 a	31.19 ± 5.06 a	30.50 ± 0.73 a
Waygo	24.70 ± 0.71 b	7.67 ± 1.44 a	32.37 ± 1.05 b	6.87 ± 0.11 b	40.44 ± 6.54 a	43.07 ± 1.23 b

Different letters indicate statistically significant differences between cultivars at the $p < 0.05$ level according to Tukey's HSD test.

Table 5. The L-ascorbic, malic, citric acid, and total acid content in leaf lettuce 'Nordice' and romaine lettuce 'Pivotal', 'Yakina', and 'Waygo' grown on a vertical farm in an aquaponics system.

Cultivar	L-Ascorbic Acid (mg 100 g^{-1} FW)	Malic Acid (mg 100 g^{-1} FW)	Citric Acid (mg 100 g^{-1} FW)	Total Acids (mg 100 g^{-1} FW)
Nordice	5.29 ± 0.82 b	327 ± 17 b	40.54 ± 2.34 a	373 ± 20 b
Pivotal	5.02 ± 0.30 b	445 ± 11 c	50.8 ± 2.97 a	501 ± 13 c
Yakina	1.09 ± 0.04 a	302 ± 3 ab	48.99 ± 2.41 a	352 ± 6 ab
Waygo	5.46 ± 0.31 b	255 ± 9 a	41.2 ± 1.21 a	301 ± 9 a

Different letters indicate statistically significant differences between cultivars at the $p < 0.05$ level according to Tukey's HSD test.

4. Discussion

4.1. Mineral Composition of Water in an Aquaponic System

The low nutrient content and high pH of the circulation water are two major issues that must be overcome to successfully combine aquaculture and hydroponics into a sustainable leafy vegetable production system [28]. Such challenges were also faced in our study of a plant factory with a recirculating aquaponic system. A pH range of 5.5 to 6.0 for hydroponically grown lettuce is preferred, which allows for maximum nutrient availability to the plant roots, and a slight increase in pH to 7.0 can significantly reduce fresh and dry weights [29]. However, the optimal pH for sturgeon aquaculture ranges between 6.5 and 8.5 [30], and nitrifying bacteria require a pH of around 7.5 for the optimal conversion of ammonia to nitrates [31]. In our study, the water in the aquaponic system contained low amounts of nitrogen, phosphorus, and potassium and sufficient levels of Ca and Mg, which came from tap water used to fill fish tanks. Graber and Junge [32] determined that an aquaponic solution contained three times less nitrogen, 10 times less phosphorus, and 45 times less potassium than a hydroponic solution. Nevertheless, they obtained a good yield, although their quality was poorer due to a lack of potassium.

4.2. Yield and Biometric Parameters

The results of our research indicate that aquaponic system production can be a sustainable alternative to the hydroponics used in plant factories, considering the yield and commercial quality of lettuce (Figures 1 and 2). In this study, we obtained a satisfactory yield of romaine lettuce of 86 g in just 21 days, which corresponds to 3.4 kg m^{-2} at a density of 40 plants m^{-2}. This may have been due to the presence of organic compounds in fish water, which can stimulate plant growth [33], the early harvest of lettuce, and consequently, the low nutritional requirements of plants in the first three weeks of growth, and a well-developed root system. The root-to-shoot fresh weight ratio for romaine-type lettuce cultivars ranged from 0.31 to 0.42, with the highest value for 'Yakina'. A similar lettuce yield was reported by Moon et al. [34] in a plant factory. They showed that the vertical farming system using hydroponics produced 37 kg m^{-2} of lettuce annually, with 14.6 growth cycles a year, and was harvested at a relatively low fresh weight of 110 g. Our previous study reported that a daily light integral equal to 17.3 mol m^{-2} per day for romaine lettuce 'Casual' and 11.5–17.3 mol m^{-2} per day for 'Elizium' allowed to obtain a high fresh weight of a head lettuce (350 and 240 g, respectively) within 30 days of cultivation in an indoor plant production facility using hydroponics with mineral fertilizers [35]. However, this rapid growth of lettuce intensified the symptoms of a physiological disorder known as "tip burn" [35,36]. Studies comparing the yield of lettuce in aquaponic systems with hydroponics are inconsistent. Some researchers have reported similar or even better yields in aquaponics than in hydroponics, despite lower concentrations of mineral nutrients [37,38]. In contrast, the growth and yield of lettuce grown in aquaponics were lower than those grown in hydroponics, and the lettuce developed leaf yellowing [39]. Among the various factors affecting the yield of aquaponic lettuce, such as the EC level, pH, nutrient profile of fish water, water quality, water temperature [40], and nutrient composition of fish feed [37,40], the type of cultivar can be considered an important factor. Our research has shown that, in terms of yield, romaine-type lettuce cultivars adapt better than leaf-type lettuce to an aquaponic environment without additional mineral nutrients in fish water.

4.3. Chlorophyll, Flavonol, and Nitrogen Balance Indices

Non-destructive techniques for assessing the nutritional status of lettuce crops provide fast and reliable results and can provide an affordable alternative to standard laboratory methods, especially in plant factories. The lowest NBI and CI indices, as well as the lowest biomass production, were recorded for the leaf-type lettuce cultivar 'Nordice', which suggests that the nitrogen content in the fish water was a factor limiting growth and development. In contrast, romaine lettuce cultivars showed higher nitrogen use efficiency, which may have been due to differing expression of nitrogen transportation and assimilation genes [41]. Becker et al. [42] showed that nitrogen treatments have a clear effect on growth characteristics, phenolic and photosynthetic compounds, nitrogen, nitrate, and carbon concentrations in leaf lettuce. The concentrations of all major flavonoid glycosides increased with decreasing nitrogen concentrations, while the concentrations of chlorophyll and β-carotene decreased. Flavonoids, as nitrogen-free secondary metabolites, are considered indicators of nitrogen availability in plants [43]. The flavonol index for all tested lettuce cultivars grown in fish water without mineral supplementation exceeded the value of 0.52 (Figure 3), while under hydroponic conditions with sufficient minerals, it did not exceed 0.23 [21].

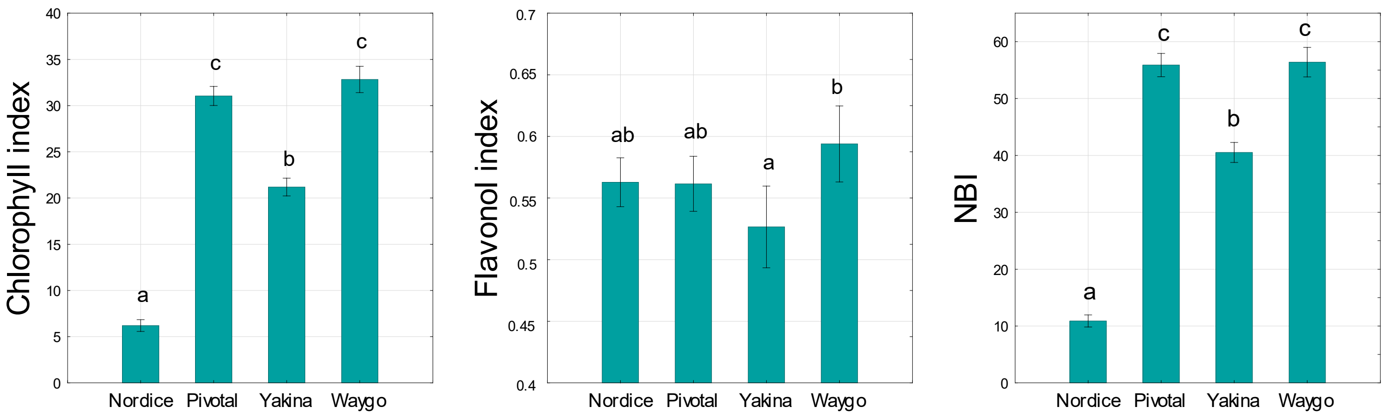

Figure 3. Chlorophyll index (CI), flavonol index, and nitrogen balance index (NBI) of three romaine lettuce cultivars ('Pivotal', 'Yakina', and 'Waygo') and one leaf lettuce cultivar ('Nordice') grown on a vertical farm in an aquaponic system. Vertical bars represent the standard errors of the means. Different letters indicate statistically significant differences between cultivars at the $p < 0.05$ level according to Tukey's HSD test.

4.4. Macro- and Micronutrients in Plants

Leafy vegetables, such as lettuce, contain a significant amount of natural nitrates, which can pose a risk to human health [44]. The European Union has set the maximum limits for nitrates in lettuce grown under cover, and these values are 5000 mg kg^{-1} in winter-grown plants and 4000 mg kg^{-1} in other seasons [45]. The concentration of nitrates in lettuce leaves grown in the aquaponic system was low (885–2221 mg kg^{-1} f.w.) and did not exceed the permissible limit imposed by the European Union, although nitrate accumulation was genotype dependent.

According to Knott's Handbook for Vegetable Growers [46], sufficient ranges of mineral nutrients for greenhouse-grown lettuce are 2.1–5.6% N, 0.5–0.9% P, 4.0–8.0% K, 0.9–2.0% Ca, 0.4–0.8% Mg, 50–200% Fe, 25–200% Mn, 5–18% Cu, 30–200% Zn, and 25–65% B. Therefore, for the romaine lettuce cultivars 'Pivotal' and 'Yakina', the concentrations of macro- and microelements in lettuce leaves were within the optimal range for macro- and microelements, despite low amounts of nitrogen, phosphorus, and potassium in the aquaponic solution. Since chemical analysis of nutrient solutions can only detect nutrients that are available in ionic form, suspended organic solids may have promoted lettuce growth. However, deficiencies of P, K, Mn, and Cu occurred in the leaves of the leaf lettuce cultivar 'Nordice' and the mini-type romaine cultivar 'Waygo', which suggests that the accumulation of some minerals depends on the genotype, growth rate, and morphological features [12]. Leaf lettuce 'Nordice' had the lowest weight, produced the fewest leaves, contained the least nitrates, and had the lowest leaf chlorophyll index (CI) and nitrogen balance index (NBI), indicating that the nutrient concentration in the aquaponic solution was a limiting factor for this type of lettuce. The solution could be supplementing aquaponic fish water with minerals, mainly phosphorus and potassium [47], or using a mineralization unit to concentrate the nutrients of aquaculture water in decoupled systems [48,49]. The lowest Ca concentration in leaves was found for the cultivar 'Waygo', which represents mini-types of romaine lettuce with closed heads. Low calcium content in young romaine lettuce leaves was probably the cause of the physiological disturbance that causes tip burn, and this phenomenon is common in romaine lettuce grown in indoor plant production systems [35].

Both our own research and the data available in the literature show a considerable variation in the chemical composition of lettuce depending on its type, variety, and method of cultivation. According to Llorach et al. [50], romaine lettuce is moderately rich in bioactive compounds, whereas iceberg lettuce is poorer, and red varieties may contain several times more phenolic compounds and vitamin C. Even a significant variation in the composition of bioactive compounds can be seen among varieties belonging to the same type of lettuce [51].

4.5. Phytochemical Content

In the current study, the chlorophyll *a* and chlorophyll *b* contents for 'Yakina' were at a similar level as those reported by Zhan et al. [52] for romaine lettuce (18 mg 100 g^{-1} and 5.8 mg 100 g^{-1}, respectively). The other varieties of romaine lettuce in our study, 'Pivotal' and 'Waygo', showed higher values of chlorophyll *a* and *b* than reported by Lopez et al. [51] and Zhan et al. [52] for the 7 tested varieties of romaine lettuce grown in greenhouse conditions (chlorophyll *a* 60–115 µg/g, which corresponds to 6.0–11.5 mg 100 g^{-1}; chlorophyll *b* 20–40 µg g^{-1}, which corresponds to 2.0–4.0 mg 100 g^{-1}) [49].

β-carotene is the dominant carotenoid in romaine lettuce [51,53]. The average carotenoid content for 7 varieties grown in greenhouse conditions was 5.8 mg100 g^{-1}, which is in the range of carotenoids in lettuces grown in the aquaponics system (4.8–6.9 mg 100 g^{-1}).

Lettuce is a vegetable that is rather poor in vitamin C compared to other vegetables. Lettuce grown in vertical farms, with the exception of the 'Yakina' cultivar, contained more than 5 mg 100 g^{-1} of this vitamin, which is a higher value than that reported by Llorach et al. [50] (2.8 mg 100 g^{-1}) and slightly less than the value reported by

Zhan et al. [52] (about 7 mg 100 g^{-1}). The analysis of organic acids confirmed that one of the dominant acids in romaine lettuce is malic acid [51].

The content of total phenolic compounds in leaf lettuce (*L. sativa*) cultivated in a different agronomic and fertilization system is between 1–2 mg/g FW (corresponding to 100–200 mg 100 g^{-1}) [54,55], which is 2–4 times higher than that found for 'Nordice' leaf lettuce in our study (50 mg 100 mg^{-1}). However, some varieties, such as *Lactuca sativa* L. cv. 'Baronet', contain significantly less phenolic compounds (10 mg 100 g^{-1}), and when subjected to high light stress, they increase the phenolic compound content up to three times [56].

Current studies on the composition of bioactive compounds have shown that romaine lettuces grown on a vertical farm in an aquaponics system achieve very good nutritional value, and the composition of phenolic compounds overlaps with that of lettuce grown traditionally in a greenhouse.

5. Conclusions

For the first time, it has been shown that a recirculating aquaponic system with sturgeon rearing can be used to efficiently produce lettuce in a commercial-scale plant factory. Ensuring optimal climatic conditions in the plant factory, including light and temperature, allowed for satisfactory yield and quality of midi-type romaine lettuce in just 21 days of cultivation. Despite the low mineral content in the aquaponic solution, the mineral content in midi-type romaine lettuce leaves was within the optimal range, and the nutritional value was similar to lettuce grown traditionally in a greenhouse. The biomass production of leaf lettuce and mini-type romaine lettuce was lower than that of midi-type romaine lettuce, and the nitrate, phosphorus, and potassium contents in the leaves were below the optimal range. Our results indicate that the effectiveness of aquaponics without additional minerals depends on the plant genotype.

Author Contributions: Conceptualization, B.M. and S.K.; methodology, B.M. and M.M.-F.; formal analysis, B.M. and M.M.-F.; investigation, B.M. and M.M.-F.; writing—original draft preparation, B.M.; writing—review and editing, B.M. and M.M.-F.; supervision, S.K.; project administration, S.K.; funding acquisition, S.K. and B.M. All authors have read and agreed to the published version of the manuscript.

Funding: The National Research Center for Research and Development in Poland, grant POIR 01.01.01-00-0579/19, "Plantlab: an innovative system of year-round production of romaine lettuce and freshwater fish in aquaponic technology".

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data are available on request.

Conflicts of Interest: The authors declare no conflict of interest.

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