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Biologically Active Compounds in Tomato Fruits Under the Application of Water–Ethanol *Spirulina, Dunaliella* and *Chlorella* Microalgae Extracts on Plants' Leaves

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Abstract: This study aimed to detect an impact of water–ethanol extracts of different microalgae species—*Spirulina platensis*, *Dunaliella salina* and *Chlorella vulgaris*—on the accumulation of bioactive compounds in tomatoes. A treatment with the corresponding ethanol solution and pure drinking water was used as a control. Tomato cultivar 'Belle' F1 (Enza Zaden) was grown in a polycarbonate greenhouse, in 25 L pots filled with a peat substrate (pH KCl 5.5). The plants were sprayed weekly from germination until the start of harvesting, in total nine times. Fruits were analysed at the stage of full ripeness. Bioactive compounds' contents such as vitamin C, titratable acidity, pH value, β-carotene, lycopene, anthocyanin, total phenols as well as total soluble solids and dry matter were analysed, and the connection between fruit mass and the taste index was determined. The influence of the tested extracts on the bioactive compounds and quality parameters of tomatoes was different, but no significant differences for most of the analysed active compounds were found, with the exception of total phenols (from 137.59 ± 1.34 to 166.93 ± 2.01 mg 100 g^{-1}) and total soluble solids (from 3.93 ± 0.12 to 4.4 ± 0.18 °Brix). In the next research, a more detailed study about the influence of the ethanol concentration on changes in biologically active compounds should be provided.

Keywords: microalgae; *Solanum lycopersicum* L.; chemical composition; lycopene

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1. Introduction

Biostimulants have emerged as a promising strategy in agriculture, aimed at enhancing the growth and yield parameters of different crops. Previous studies have highlighted microalgae-based biostimulants as a favourable, ecological and sustainable approach for increasing agricultural crops' yield and overall rural sustainability [1]. Microalgae extracts are recognised as biostimulants due to their bioactive compounds, high metabolic activity and the capacity to promote plant growth and development [2–4]. Microalgae are characterised by their rapid growth, high photosynthetic efficiency and lower natural resource demands compared to conventional agricultural crops [5,6]. In addition, they are considered a valuable nutritional resource that can decrease the need for extensive farmland, thereby mitigating the environmental effect of agricultural production while promoting population health [7]. Additionally, microalgae find applications in various industries, including aquaculture, poultry [8], cosmetics and pharmaceuticals [5,8–11], as a source of biofuels, for food and feed supplements [8,12] and as biological fertilisers, including micro- and macro elements [12].

Microalgae, photosynthetic microorganisms among the earliest organisms of life on the planet, remain relatively unexplored [13]. Scientists suggest that there are a hundred thousand to many millions of microalgae species, though only about seventy-three thousand have been identified, with a small proportion cultivated on an industrial scale for

marketable intentions [14]. The cultivation of microalgae has been a significant focus in biotechnology recently [12]. Microalgae are known for producing a wide range of antioxidant molecules, including polyphenols, tocopherols, phycobilins, carotenoids (β-carotene, lutein, astaxanthin) and essential fatty acids [5,9,15–18], along with the other phenolic compounds and chlorophyll a, b pigments [12,19,20], and they are particularly noteworthy for their antioxidant properties and health benefits [21]. Microalgae contain biologically active compounds, for example vitamins and minerals, saccharides, lipids, proteins and pigments [9,22]. Owing to the different chemical characteristics of microalgae, they can be used as nutritional supplements or a source of natural food colourants [8]. The antiradical activity of microalgae is strongly associated with their biologically active compounds. Microalgae can improve the nutrition content of traditional food products, thereby definitely impacting the healthiness of people and animals [8]. Though less studied, scientists suggest the potential of microalgae biomass as slow-release organic fertiliser [12], promoting vegetable growth and increasing crop yields. For instance, the cultivation of tomatoes and persimmons with microalgae resulted in improved fruit quality with higher carotenoid and sugar contents [12,23]. Regarding croplands, researchers have found that recovering nutrients using microalgae cultivation provides higher value than directly applying waste streams [12,24].

Tomato (*Solanum lycopersicum* L.) is a commercially important vegetable crop that is cultivated and consumed fresh and processed. It is one of the most used vegetables in the diets and thus the major source of biologically active compounds [25–27], with a high taste quality and nutritional value [28–30]. In recent years, tomatoes have seen a surge in popularity, largely due to their rich biologically active compounds, for example phenolic compounds, carotenoids, anthocyanins and flavonoids, along with essential nutrients, like sugars and essential oils. Tomato fruits offer a unique flavour and taste, along with significant medicinal value and health benefits [31,32].

There is a lack of information about the effectiveness of algae extracts, especially ethanol-based, on the biologically active compounds of tomatoes. Foliar treatment is generally recommended because of the fact that the physical and chemical properties of soils can slow down the uptake of macro- and micronutrients [1]. A group of researchers from Algal Biotechnology Unit, Egypt [33], demonstrated that under treatment with different microalgae, the physiological effect of irrigation on tomato seedlings can be counteracted by improving the ability of the roots to absorb more nutrients in comparison with control plants. Another study demonstrated that tomatoes treated with 0.5% *Spirulina platensis* can extend the shelf life of tomatoes by up to 52 days [34]. On the other hand, when tomatoes were treated with *Chlorella vulgaris*, fruits could be stored for up to 45 days at room temperature, which was significantly more than control tomatoes and thus can help to solve tomato-storage problems [33].

This study aimed to evaluate biologically active compound accumulation in tomatoes under the application of water–ethanol *Spirulina platensis*, *Dunaliella salina* and *Chlorella vulgaris* microalgae-based extracts on plants' leaves.

2. Materials and Methods

2.1. Preparation of the Microalgae Extractions

Microalgae *Spirulina platensis*, *Chlorella vulgaris* and *Dunaliella salina* were grown autotrophically in 14 L closed-system photo-bioreactors (VariconAqua Solutions Ltd., Worcester, UK). The obtained biomass (12 L) from the photo-bioreactor was poured into a bucket and then put in a refrigerator (+4 °C) to allow it to settle for 24 h. Then, the upper layer (8–9 L) was gently aspirated and distributed in tubes (preferably with a volume of 250 mL) and centrifuged at 8000 rpm for 5 min. After that, the supernatant was poured off, 200 mL of distilled water was added for rinsing, the solution was mixed and centrifuged again and then, process was repeated. The precipitate was dried in the thermostat at +45 to +50 °C. The obtained biomass was pulverised with a pestle.

The initial extract was prepared by adding 1 L of water–ethanol solution (3:1) to 300 g of an appropriate microalgae powder (previously prepared and pulverised in a pestle). After mixing, the prepared solution was kept for 24 h at room temperature for extraction. The prepared extract (Figure 1) was used at a 10% and 20% concentration by dissolving it in water.

Figure 1. Water–ethanol microalgae-based extracts prepared for the trial: *Spirulina platensis* (on the **left**), *Dunaliella salina* (in the **centre**) and *Chlorella vulgaris* (on the **right**).

The extraction conditions, as well as two concentrations, 10% and 20% (v/v), used in our research, were chosen after the data analysis of pre-experimental research in 2021. For example, in this pre-research, a concentration of extracts less than 10% showed no effect on the tested plants. Table 1 illustrates the quality parameters per 30 L of prepared water–ethanol extracts.

Table 1. Quality parameters of water–ethanol microalgae-based extracts.

Parameters	*Spirulina platensis*	*Dunaliella salina*	*Chlorella vulgaris*
Organoleptic properties	Dark brown, homogeneous suspension, turbidity and sediment are acceptable, with characteristic smell and taste.	Orange, homogenous suspension, turbidity and sediment are acceptable. With a characteristic smell and taste.	Greenish-brown, homogeneous suspension, turbidity and precipitates are acceptable. The smell and taste were appropriate for the species.
Physico-chemical properties:			
density at 20 °C, g cm^{-3}	0.974	1.058	0.971
Ethanol concentration, %	25	25	25
pH, 20 °C	6.8	5.6	6.4
Content of the non-volatile part, %	1.7	27	1.3
Microbiological agents:			
total number of microorganisms, *CFU*, 1 mL (1 g)	$<10^4$	$<10^4$	$<10^4$
Yeast, CFU, 1 mL (1 g)	<50	<50	<50
Salmonella sp., 25 g	No present	No present	No present
Coliforms, 1 mL (1 g)	No present	No present	No present
Storage conditions and shelf-life	8–25 °C, 36 months	8–25 °C, 36 months	8–25 °C, 36 months

In this study, water–ethanol extracts as innovative products of the microalgae, were tested. Initially, the idea of the project (see section–Funding) was to find algae-based fertilisers and biostimulants, easy for use in commercial farms, constant based on quality parameters and with a long shelf-life. Therefore, water–ethanol extraction instead of pure water extraction was used. Based on the quality parameters, the obtained extracts were in accordance with the defined indicators, including a long shelf-life for at least 36 months.

2.2. Plant Material and Sampling

Investigations were conducted in a polycarbonate greenhouse at the Latvia University of Life Sciences and Technologies, Faculty of Agriculture and Food technology, Institute of Soil and Plant Sciences, Laboratory of Horticulture and Beekeeping. Seeds of tomato cultivar 'Belle' F1 (Enza Zaden) were sown on the 1 July 2023 in plastic cassettes with a peat substrate (pH KCl 5.5, producer Laflora Ltd., Jelgava, Latvia). On the 18 July the seedlings were replanted in 1 L pots, except in August 2023, at the age of 35 days, in 25 L pots with the same peat substrate. The plants were sprayed on their leaves weekly from the time of seedling replanting until the start of harvesting, in total for nine times with the solution of water–ethanol extractions of *Spirulina platensis*, *Dunaliella salina* and *Chlorella vulgaris*. During the experiment, each plant was sprayed with the same amount of the extraction from 3 (for seedlings) to 15 (for mature plants) seconds, according to development's stage. Two concentrations of these extracts (10% and 20% v/v) were compared to treatments with corresponding ethanol solutions as controls (2% and 4% v/v), as well as a control treatment with drinking water. Nine plants per treatment were used.

During the experiment, plant maintenance (irrigation, fertilisation, phytosanitary measures) was provided regularly. The plants were pruned using a traditional scheme. The tomato plants were fertilised once a week with a solution of YaraTera mineral fertilisers (according to the producer's recommendations): during the seedling growing period–with Kristalon Green (NPK 18-18-18), with microelements; after seedling replanting until the reproductive phase, with Kristalon Yellow (NPK 13-40-13) with microelements; and during the reproductive phase, with Kristalon Red (NPK 12-12-36) with microelements. Automatic ventilation and additional lighting, provided by high-pressure sodium lamps, were carried out based on necessity. The yield was harvested 13 times, once a week, from the end of September (30/09) to the end of December (23/12), at the fully ripe stage (Figure 2).

Figure 2. Maturity stages of tomato: from mature green (**right**) to fully ripe (**left**), trial photo.

2.3. Determination of Vitamin C Content

The vitamin C content was determined titrimetrically using 2.6-dichlorophenol-indophenol [35]. For the analysis, 3 ± 0.001 g of tomato fruit was quantitatively moved into 100 mL tubes, followed by the addition of 50 mL of a 1% HCl and 5% H_3PO_4 solution (1:1 *v*/*v*) and thorough mixing; 30 min later, the solution was filtered through filter paper. Next, 10 mL (V_a) of filtrate was titrated with a 0.0005 M solution of 2.6-dichlorophenol-indophenol (V_{titr}). The vitamin C content (mg 100 g^{-1}) was calculated using Equation (1).

$$m = \frac{v_{titr} \times 0.044 \times V_t \times 100}{V_a \times \text{weight}} \quad (1)$$

where

m is the content of vitamin C;

V_{titr} is the volume of 2.6-dichlorophenol-indophenol used for titration, mL;

0.044 is the amount of ascorbic acid required to reduce 1 mL of a 0.005 M 2.6-dichlorophenol–indophenol solution, expressed in mg;

V_t is the total filtrate volume, mL;

V_a is the volume of filtrate (10 mL);

weight–weighed amount of plant material.

2.4. Determination of Titratable Acidity (TA)

The titratable acidity was determined titrimetrically with a sodium hydroxide solution [36]. A 3 ± 0.001 g sample of tomatoes was quantitatively moved into a 20 mL tube, followed by the addition of 20 mL of distilled water and then shaking and centrifugation (speed 6000 rpm/rcf) for 3 min. To determine the titratable acidity, 5 mL of the supernatant was titrated with 0.1 M NaOH using phenolphthalein as the indicator. Equation (2) was used for the calculations.

$$TA = \frac{V_{NaOH} \times V_t}{V_s \times m} \quad (2)$$

where

TA is the titratable acidity;

V_{NaOH} is the volume 0.1 M NaOH;

V_t is the total volume (20 mL);

V_s is the sample volume (5 mL).

Titratable acidity was calculated as grams of $C_6H_8O_7$ per 100 g of the fresh weight (FW) tomato fruit sample.

2.5. pH Measurement

The pH value was measured using the standard method LVS ISO 5542:2010 pH Meter Jenway 3520.

2.6. Determination of β-Carotene and Lycopene Contents

To determine β-carotene and lycopene concentrations, 0.5 ± 0.001 g of tomatoes was quantitatively moved into a tube, and then, 10 mL of tetrahydrofuran (THF) was added [37]. Tubes were closed and left at a temperature +20 ± 1 °C for 30 min with mixing then separated in a centrifuge for 3 min (speed 6000 rpm/rcf). The absorbance was measured at wavelengths of 453, 505, 645, and 663 nm with the spectrophotometer UV-1800, Shimadzu Corporation, Japan. Lycopene and β-carotene contents (mg 100 g^{-1}) were calculated according to the Equations (3) and (4).

$$C_{car} = (0.216 \times A_{663}) - (1.220 \times A_{645}) - (0.304 \times A_{505}) + (0.452 \times A_{453}) \quad (3)$$

$$C_{lyc} = (0.046 \times A_{663}) + (0.204 \times A_{645}) + (0.372 \times A_{505}) - (0.081 \times A_{453}) \quad (4)$$

where

A_{663}, A_{645}, A_{505} and A_{453} are the absorbance at specified spectrophotometer wavelengths [38].

2.7. Determination of Anthocyanin Content

The anthocyanin content was determined using the spectrophotometer UV-1800 (Shimadzu Corporation, Kyoto, Japan) at wavelength of 540 nm [39,40]. Here, 3 g of tomatoes was quantitatively moved into a tube, and then, 20 mL of a 1.5 M HCl and ethanol solution (85:15 v/v by volume) was added, and the sample was shaken for 30 min and separated in a centrifuge for 3 min (speed 6000 rpm/rcf). Samples were diluted until the absorbance coefficient was between 0.80 and 0.60. The anthocyanin content (mg 100 g^{-1}) was calculated according to Equation (5) [41].

$$C = \frac{A \times v \times d \times 1000}{980 \times m} \quad (5)$$

where

C is the anthocyanin content;
A is the absorption coefficient;
v is the extraction volume;
d is the dilute solution;
m is the mass of the sample.

2.8. Determination of Total Phenol Content

The total phenol content was assessed using a modified spectrophotometric method, with readings taken with a spectrophotometer UV-1800. For the determination of total phenols, 3 ± 0.001 g of tomatoes was quantitatively moved into a tube, and 20 mL of a hydrochloric acid solution–water–methanol (1:20:79 $v/v/v$) solution added, and then the sample was shaken for 30 min and centrifuged for 3 min (speed 6000 rpm/rcf). The absorption was measured at the wavelength of 320 nm (A_{320}). The content of total phenolics (mg GAE g^{-1}) was calculated using Equation (6).

$$m = \frac{A_{320} - 0.09}{0.009 \times m_{weight}} \quad (6)$$

where

m is the total phenol content;
A_{320} is the absorption determined experimentally at a 320 wavelength;
m_{weight} is the sample weight, g;
m is the phenol content in the plant material [42].

2.9. Determination of Dry Matter (DM) and Total Soluble Solids (TSSs)

Dry matter was determined by drying samples in the thermostat at 60 °C.

The TSS °Brix content in tomato fruits was determined with a high-precision digital handheld refractometer (A.KRÜSS) DR301-95, according to the standard method ISO 2173:2003.

2.10. Determination of Tomato Fruit Mass and Taste Index

In order to determine the mean weights and the dimensions of tomato fruits, fruits of each sample were counted and weighed during harvesting using an electric balance with a precision of ±0.001 g.

The taste index was calculated using the soluble solid content and the acidity [43,44], according to Equation (7).

$$TI = \frac{TSS}{20 \times TA} + TA \quad (7)$$

where

TSS is the total soluble solids, °Brix;

TA is the titratable acidity [45].

Correlations between fruit mass and the taste index in tomato fruits were also analysed.

2.11. Statistical Analysis

Data analysis was performed using SPSS 21. For one-Way-ANOVA, Post Hoc Multiple Comparisons, Scheffe Post Hoc Tests for Multiple Comparisons and Homogeneous Subsets of variance tests were conducted to determine the significance of differences, $p < 0.05$; $\alpha = 0.05$ was considered statistically significant. Results were expressed as means ± standard deviations (S.D.s).

Figure 3 depicts the general scheme of the study.

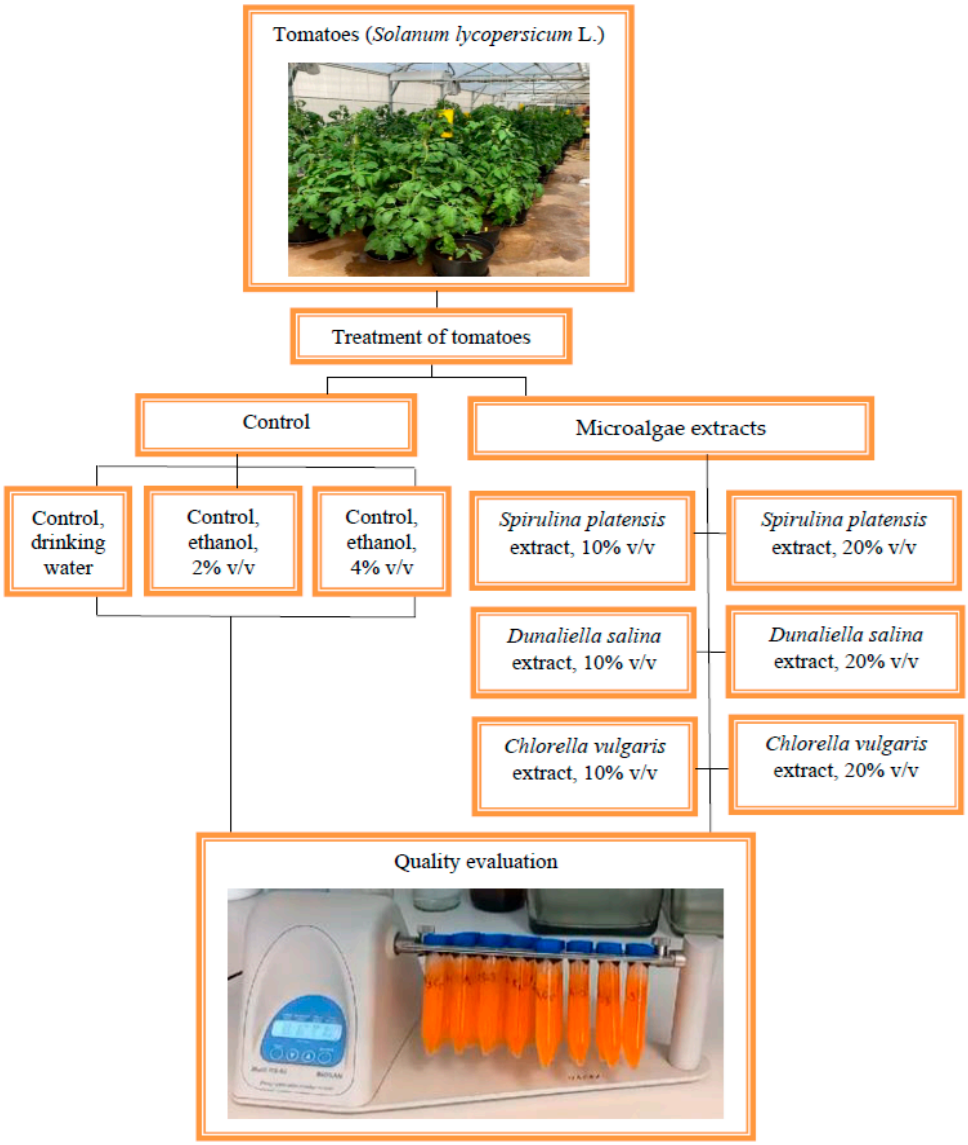

Figure 3. General scheme of study.

3. Results

The statistical analysis confirmed that varying the concentrations of the *Spirulina platensis*, *Dunaliella salina* and *Chlorella vulgaris* extracts did not alter the vitamin C content in tomatoes significantly ($p = 0.304$; $\alpha = 0.05$). The highest vitamin C content, observed in this study, was 18.88 ± 0.73 mg 100 g^{-1} of (FW) in tomatoes treated with the *Dunaliella salina* extract,

10% *v*/*v*. In turn, with the control, ethanol, 4% *v*/*v*, *Spirulina platensis* extract, 20% *v*/*v* and *Chlorella vulgaris* extract, the 20% *v*/*v* treated tomato vitamin C content decreased (p = 1.000; α = 0.05), respectively, to 15.68 ± 0.56; 15.68 ± 0.94 and 15.47 ± 0.83 mg 100 g^{-1} (FW) (Table 2).

Table 2. Vitamin C, titratable acidity and pH value in tomato fruits.

Type of Extract	Concentration, %	Vitamin C, mg 100 g^{-1} FW	* Titratable Acidity, g 100 g^{-1} FW	pH
Chlorella vulgaris	10	17.47 ± 0.82 [a]	0.32 ± 0.03 [a]	4.30 ± 0.11 [a]
	20	15.47 ± 0.83 [a]	0.28 ± 0.01 [a]	4.27 ± 0.12 [a]
Dunaliella salina	10	18.55 ± 0.73 [a]	0.28 ± 0.01 [a]	4.25 ± 0.15 [a]
	20	17.21 ± 0.58 [a]	0.25 ± 0.02 [a]	4.25 ± 0.10 [a]
Spirulina platensis	10	16.17 ± 0.98 [a]	0.32 ± 0.02 [a]	4.23 ± 0.16 [a]
	20	15.68 ± 0.94 [a]	0.27 ± 0.02 [a]	4.22 ± 0.10 [a]
Control, ethanol	2	16.32 ± 0.64 [a]	0.45 ± 0.03 [a]	4.24 ± 0.11 [a]
	4	15.68 ± 0.56 [a]	0.31 ± 0.03 [a]	4.25 ± 0.14 [a]
Control, drinking water	-	17.96 ± 0.89 [a]	0.33 ± 0.02 [a]	4.21 ± 0.14 [a]

* Results expressed as g 100 g^{-1} of citric acid. Means with the same letter are not significantly different (p > 0.05; α = 0.05), Scheffe test.

The titratable acidity (TA) value is an important indicator of tomato fruit quality. Tomatoes, known for their acidic taste, have a pH largely determined by their acid content, which also contributes to the flavour of tomato products [46]. The titratable acidity in the tomato samples was from 0.25 ± 0.02 to 0.45 ± 0.03 g 100 g^{-1} (FW), which indicates an acidic environment in the fruit (Table 2). There was no significant difference between the samples (p = 0.637; α = 0.05). The highest titratable acidity content of 0.45 ± 0.03 g 100 g^{-1} (in fresh weight, FW) was found in fruits under the control, ethanol, 2% *v*/*v*. Lower indicators were gained in tomatoes treated with the *Dunaliella salina* extract, 20% *v*/*v*, *Spirulina platensis* extract, 20% *v*/*v*, respectively, at 0.25 ± 0.02 and 0.27 ± 0.02 g 100 g^{-1} (FW).

The pH value, which reflects the concentration of hydrogen ions and indicates the acidity level [47], is shown in Table 2. The pH value of tomatoes was not significantly influenced (p > 0.05; α = 0.05) by the type and concentration of microalgae species applied, as well as their interaction. The maximum values of pH (4.30 ± 0.11) were found in tomato fruits treated with the *Chlorella vulgaris* extract, 10% *v*/*v*, while the minimum value of pH (4.21 ± 0.14) was detected in the control variant with drinking water.

Tomato fruits contain water-soluble components, like fructose, glucose, sucrose and pectin [47]. Soluble solids are the contents of total soluble sugars, which are determined refractometrically in °Brix units. The soluble solids are another major quality parameter for vegetables. It is known that the soluble solid content changes as a tomato fruit ripens. Table 3 shows total soluble solids (°Brix), as well as the dry matter (%), in tomatoes that were treated with different microalgae extracts. Our research proved that the content of soluble solids in tomatoes was significantly influenced by the extract application, as well as their interaction. The maximum contents of the total soluble solids were 4.4 ± 0.18 °Brix (control, drinking water) and 4.29 ± 0.05 °Brix (*Dunaliella salina* extract, 20% *v*/*v*) (p = 0.834; α = 0.05), while the minimum content of the total soluble solids was 3.93 ± 0.12 (*Chlorella vulgaris* extract, 20% *v*/*v*) (p < 0.05; α = 0.05).

Table 3. Total soluble solids, lycopene, β-carotene, anthocyanins and total phenols in tomato fruits.

Type of Extract	Concentration, %	Total Soluble Solids, °Brix	Lycopene, mg 100 g^{-1} FW	β-Carotene, mg 100 g^{-1} FW	Anthocyanins, mg 100 g^{-1} FW	Total Phenols, mg 100 g^{-1} FW
Chlorella vulgaris	10	4.09 ± 0.02 ^{abc}	0.68 ^{a}	2.85 ^{a}	0.11 ± 0.01 ^{a}	145.96 ± 2.04 ^{ab}
	20	3.92 ± 0.03 ^{a}	0.92 ^{a}	3.48 ^{a}	0.12 ± 0.01 ^{a}	145.49 ± 2.25 ^{ab}
Dunaliella salina	10	4.14 ± 0.01 ^{bc}	0.65 ^{a}	2.62 ^{a}	0.14 ± 0.02 ^{a}	142.22 ± 2.02 ^{ab}
	20	4.29 ± 0.01 ^{cd}	0.81 ^{a}	3.23 ^{a}	0.09 ± 0.01 ^{a}	143.33 ± 1.22 ^{ab}
Spirulina platensis	10	4.01 ± 0.04 ^{ab}	0.84 ^{a}	3.39 ^{a}	0.12 ± 0.02 ^{a}	144.21 ± 1.87 ^{ab}
	20	4.17 ± 0.02 ^{bc}	0.70 ^{a}	2.89 ^{a}	0.11 ± 0.01 ^{a}	142.23 ± 1.33 ^{ab}
Control, ethanol	2	3.99 ± 0.01 ^{ab}	0.60 ^{a}	2.62 ^{a}	0.09 ± 0.01 ^{a}	137.59 ± 1.34 ^{a}
	4	4.11 ± 0.04 ^{abc}	0.82 ^{a}	3.41 ^{a}	0.07 ± 0.01 ^{a}	142.21 ± 1.53 ^{ab}
Control drinking water	-	4.4 0± 0.02 ^{d}	0.98 ^{a}	4.07 ^{a}	0.42 ± 0.04 ^{a}	166.93 ± 2.01 ^{c}

Means with the same letter are not significantly different ($p > 0.05$; $\alpha = 0.05$), Scheffe test.

Carotenoids are red, orange and yellow lipophilic pigments, which help to protect plants from photooxidative harm [26]. In turn, lycopene is an important carotenoid in tomatoes, which is responsible for their red colour [48,49]. This bright red pigment and phytochemical is also found in other green and red vegetables [50].

The lycopene content in the tomatoes was not significantly influenced by different microalgae species (Table 3), their application concentrations or their interaction ($p = 0.156$; $\alpha = 0.05$). The highest contents of lycopene, 0.98 ± 0.10 and 0.92 ± 0.06 mg 100 g^{-1} (FW) ($p = 1.000$; $\alpha = 0.05$), were determined in tomatoes treated with the control, drinking water and *Chlorella vulgaris* extract, 20% v/v, respectively, while the lowest contents of lycopene (0.60 ± 0.03 mg 100 g^{-1}, 0.66 ± 0.05 and 0.68± 0.02 mg 100 g^{-1} (FW), were noted in variants with the control, ethanol, 2% v/v; *Dunaliella salina* extract, 10% v/v, and *Chlorella vulgaris* extract, 10% v/v, respectively. The Table 3 also shows data on the β-carotene contents in tomato fruits, revealing no significant treatment effects ($p = 0.118$; $\alpha = 0.05$). The maximum content of β-carotene, 4.07 ± 0.19 mg 100 g^{-1} (FW), was found in control plants with drinking water, and the minimum contents of β-carotene, 2.62 ± 0.14 and 2.62 ± 0.18 mg 100 g^{-1} (FW), were found in tomato fruits under the control, ethanol, 2% and *Dunaliella salina* extract, 10% v/v, respectively.

Polyphenols are secondary metabolites produced by the plant against biotic and abiotic stresses, as they are involved in the processes of crop vegetation and growth [51]. Anthocyanins, pigments that can be red, purple or blue in colour, are primarily found in flowers, vegetables and fruits. These compounds are natural, water-soluble pigments. Anthocyanin appears as a red pigment in an acidic environment and as a blue pigment in alkaline conditions. No significant differences were observed in the anthocyanin content in tomato fruits among the treatments with the different microalgae species and control samples ($p = 0.708$; $\alpha = 0.05$) (Table 3). For all samples, the content of anthocyanins ranged from 0.07 to 0.42 mg 100 g^{-1} (FW). The highest content of anthocyanins, 0.42 ± 0.04 mg 100 g^{-1}, was found in the tomato samples under the control with drinking water ($p > 0.05$; $\alpha = 0.05$) (FW), and the lowest anthocyanin concentration was found in the control of ethanol, 4% v/v 0.09 ± 0.01 mg 100 g^{-1} (FW) ($p > 0.05$; $\alpha = 0.05$). Data about the total phenols in tomatoes are represented in Table 3. The contents were significantly affected by the different species of microalgae and their interaction ($p < 0.05$; $\alpha = 0.05$). The maximum content of 166.93 ± 2.01 mg 100 g^{-1} (FW) was found in tomato fruits under the control with drinking water ($p = 0.04$; $\alpha = 0.05$), whereas the minimum content of total phenols, 137.59 ± 1.34 mg 100 g^{-1} (FW), was found under the control, ethanol, 2% v/v.

The content of dry matter (DM) in the tomato fruits presented no significant differences between different microalgae species ($p = 0.785$; $\alpha = 0.05$) (Table 4). In this research, the DM varied from 3.49% to 4.91%.

Table 4. Dry matter, taste index and tomato fruit mass.

Type of Extract	Concentration, %	Dry Matter, %	Taste Index	Tomato Fruit Mass, g		
				In Average	Minimum	Maximum
Chlorella vulgaris extract	10	4.64	0.33	94.85 [a]	34.7	188.75
	20	4.59	0.33	104.78 [a]	55.27	195.21
Dunaliella salina extract	10	4.62	0.37	129.33 [a]	55.71	273.75
	20	4.88	0.39	102.52 [a]	39.45	218.00
Spirulina platensis extract	10	4.91	0.57	101.66 [a]	39.25	185.73
	20	4.58	0.54	116.15 [a]	40.34	213.28
Control, ethanol	2	4.81	0.38	95.11 [a]	45.08	170.00
		4.61	0.34	115.21 [a]	37.89	325.76
Control, drinking water	-	3.49	0.31	92.49 [a]	43.05	200.01

Means with the same letter are not significantly different ($p > 0.05$; $\alpha = 0.05$), Scheffe test.

The maximum fruit mass (Table 4) (325.76 g) was observed in the control variant with ethanol at 4% v/v, but the taste index with it was only 0.34, whereas the minimum fruit mass (34.7 g) was observed for the variant with the *Chlorella vulgaris* extract at 10% v/v with a taste index of 0.33. The highest taste index was observed for both variants with the *Spirulina platensis* extract: 0.57 under 10% v/v and 0.54 under 20% v/v (Table 4). For these variants, the average fruit mass was 101.66 g and 116.15 g, respectively. The minimum taste index was observed for variants with drinking water (0.31 value), and the average fruit mass was 92.49 g for this variant. On average, the highest result (129.33 g) in fruit mass was observed for the variant with the *Dunaliella salina* 10% v/v extract, while the lowest average fruit mass (92.49 g) was noted under the control with drinking water (Table 4). The difference between the average tomato fruit mass of the treatments was not statistically significant ($p = 0.673$; $\alpha = 0.05$). The *Spirulina platensis* extracts increased the taste index.

4. Discussion

Vitamin C is a major and important phytonutrient in tomatoes. It is crucial to minimise the duration of the vitamin C extraction and analysis process because it is highly sensitive to light and oxygen [52]. Scientists from Latvia mention [53] that the vitamin C content is often dependent on the environment or stress factors, such as temperature, light, moisture or the use of various plant-protection products and fertilisers. In the present study, the content of vitamin C in tomatoes was between 15.47 and 17.96 mg 100 g^{-1} (FW). As non-significant differences were observed in our research, this parameter was not affected by microalgae extracts. Researchers from Romania describe that the content of vitamin C in tomatoes was from 15.5 to 20.7 mg 100 g^{-1} (FW) [54]. In addition, Slovak researchers' studies suggest that the average content of vitamin C in tomatoes is between 16.03 and 21.51 mg 100 g^{-1} (FW) [55]. In turn, other studies by Latvian scientists reported lower results, where the content of vitamin C in tomatoes was 4.14–8.07 mg 100 g^{-1}, 11.23–18.43 mg 100 g^{-1} and 9.9–15.4 mg 100 g^{-1} (in fresh weight) [56,57].

Based on scientists from Slovakia [55], riper tomatoes have a higher pH value. On the other hand, the acidity increases during ripening up to the maturity stage, after which it decreases. This can describe the pH results (4.21–4.30) in the current study.

It is mentioned that the composition of carotenoids can vary quantitatively and qualitatively, and it can be affected by the cultivar, storage conditions and duration [58,59], as well as the climate, season, geographic location and maturity phase [60]. Similar results as in our research regarding the lycopene content in tomatoes were found in other scientific literature analysed in Latvia, where data varied from 0.07 to 27.11 mg 100 g^{-1} (FW) [36,61]. Regarding β-carotene, similar results were found in the scientific literature about tomatoes tested in Latvia: 0.04–11.73 mg 100 g^{-1} [34,58]. Slovak scientists determined the lycopene content in red tomatoes from 1.16 to 5.57 mg 100 g^{-1} and 0.88 to

4.20 mg 100 g^{-1} (FW) [62,63]. The bioactive antioxidant potential of lycopene is comparatively higher than that of β-carotene [64,65]. Researchers [12] accepted that different microalgae species extracts influence higher or lower concentrations of carotenoids in tomato fruits. Researchers from Latvia [36] reported that comparing biochemical compounds can be challenging in connection with the significant influence of farming conditions, including the varieties and harvest period. Furthermore, the content of these compounds increases from the mature green stage to the red stage [66,67]. Based on our results, it is possible to conclude that the composition of carotenoids was not affected by microalgae extracts.

Soluble solids affect the sweetness of vegetables [68] and length of the storage period, as well as quality characteristics [69]. Based on a group of researchers from Belgium [12], microalgae fertilisation improves the quality of the tomato fruits obtained, thereby increasing the sugar content in tomatoes. Scientific literature mentions that the dry matter content in tomatoes was between 5.42 and 8.25%, but the content of soluble solids was from 1.11 to 10.2 °Brix [35,36,54]. The taste index of Latvian tomatoes described in the available articles varied between 0.95 and 1.38 values [36,62] and between 0.95 and 1.26 values [35]. The different values, found in our study (0.33–0.57), can be explained by the fact that that our tomatoes were grown in the autumn–winter season, not in the summer production cycle, as in the previous studies. On the other hand, there were different data in another article, where the taste index was from 0.34 to 0.53 values [54], similar to those in our study.

The results obtained for fruit mass are consistent with previous findings [35]. Some researchers [70,71] reported fruit masses ranging from 66 to 158 g and from 107 to 185 g, respectively, which are lower than the results of our study. In the Latvian scientist's study [35], it was reported that customers often consider both the size and colour of tomato fruits. When comparing the tomato fruit taste index and fruit mass, it is proven that the taste index is associated with either larger or smaller tomatoes. Moreover, researchers from China [72] concluded that the tomato size can also be affected by infrequent watering, which can significantly reduce the fruit size, making them more susceptible to additional stress. Thus, it is important to implement a balanced water-management strategy for tomatoes to ensure the best quality of agricultural production. Scientists Sousa et al. from Portugal [2] have proven that microalgae-based bio-stimulants stimulate cucumber and tomato sees germination, which could possibly increase the fruit mass as well, and the others [9,12] claimed that increasing the biomass promotes plant growth and enhances both crop yields and quality. Additionally, scientists [12] have reported that nutrient recovery from microalgae farming could recycle into microalgae green fertilisers, thus improving the high-quality food product market value.

A group of Italian and Spanish scientists concludes that the genotype, environment and treatment are highly significant for many traits, but still, the genotype (cultivar) usually is the main source of variation [73]. In our results, adverse effects on tomato fruit quality were often observed in the treatments and controls using ethanol, which was worse than those treated with drinking water. However, these differences were not significant in our research. According to the older scientific literature, *Solanum lycopersicum* is not sensitive to the ethanol concentration, but on the other hand, ethanol interferes with ethylene production, which may affect the fruit-ripening rate [74]. Some articles say that as the ethanol percent concentration increases, there is a high probability that the ripeness process of the fruit will slow down [75]. Therefore, it is necessary to compare water and water–ethanol algae-based extracts to understand the role of the ethanol concentration in decreasing the content of some biochemical compounds. If the concentration of ethanol in the extracts is indeed too high and the influence on tomatoes' biochemical parameters is negative (in comparison with water-based extracts), it would be possible to conclude that the potential of algae-based material is much higher than the water–ethanol extract can provide.

5. Conclusions

The maximum vitamin C content, 18.55 ± 0.73 mg 100 g^{-1} (in fresh weight), was determined in the tomato fruits treated with the *Dunaliella salina* 10% *v*/*v* extract. The maximum titratable acidity was found in tomatoes treated with control, 2% *v*/*v* ethanol, 0.45 ± 0.03 (in fresh weight). The maximum value of pH 4.30 ± 0.11 was found in the tomato fruits treated with the *Chlorella vulgaris* 10% *v*/*v* extract. The maximum content of β-carotene, 4.07 ± 0.19 mg 100 g^{-1} (in fresh weight), was determined in the fruits of control plants treated with drinking water. The highest contents of lycopene, 0.98 ± 0.10 and 0.92 ± 0.06 mg 100 g^{-1} (in fresh weight), were determined in the tomato fruits treated with drinking water and the *Chlorella vulgaris* 20% *v*/*v* extract, respectively. The anthocyanin content was higher in the tomato samples from the control variant with drinking water 0.42 ± 0.04 mg 100 g^{-1} (in fresh weight). The maximum total phenol content of 166.93 ± 2.01 mg 100 g^{-1} (in fresh weight) was found in tomatoes treated with drinking water. The maximum content of total soluble solids was 4.4 ± 0.18 °Brix under the control with drinking water and 4.29 ± 0.05 °Brix under treatment with the *Dunaliella salina* 20% *v*/*v* extract, which were significantly higher ($p < 0.05$; $\alpha = 0.05$) compared to the other samples.

The dry matter content determined in the tomatoes varied from 3.49% to 4.91%. The highest taste index results (0.57 and 0.54) were with the *Spirulina platensis* 10% *v*/*v* and 20% *v*/*v* extracts, respectively. The maximum fruit mass (325.76 g) was observed in the control variant with 4% *v*/*v* ethanol. On average, the highest result (129.33 g) in fruit mass was observed for the samples with the *Dunaliella platensis* 10% *v*/*v* extract.

Our study showed that the influences of microalgae-based bio-stimulants of *Spirulina platensis*, *Dunaliella salina* and *Chlorella vulgaris* on the quality parameters of tomatoes are different, but not very effective. A statistically significant influence was observed only for the total phenol content and total soluble solids. As a result, we can propose a more detailed study of the influence of the ethanol concentration on changes in biologically active compounds in the next research.

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