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Article

Biologically Active Compounds of Tomato Fruits Affected by Application of *Spirulina*, *Dunaliella* and *Chlorella* Microalgae Extracts

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Abstract: The present study aimed to investigate the effect of solution of ethanol extractions of different microalgae species: *Spirulina* sp., *Dunaliella* sp. and *Chlorella* sp. on the accumulation of biologically active compounds in tomato fruits. Ethanol solution as well as treatment with drinking water were used as a control. Tomato cultivar 'Belle' F1 (Enza Zaden) was grown in 25 L pots, in peat substrate (pH_{KCl} 5.5, producer Laflora LTd), polycarbonate greenhouse. Chemical composition – total carotenoids, lycopene and β-carotene content, total phenolics and anthocyanins, organic acids, vitamin C as well as dry matter, pH and soluble solids content – was determined in tomato fruits at the stage of full ripen. The influence of tested extracts on biologically active compounds on quality parameters of tomatoes was different, but no negative effect was observed. As only pH values as well as total phenol content and total soluble solids had statistically significant differences, results can be described with genetical stability of the cultivar.

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

1. Introduction

The use of biostimulants in agriculture has emerged as a promising strategy to improve crop growth and yield. Microalgae extracts as bio-stimulants are known because of their bioactive compounds, high metabolic activity as well as ability to enhance plant growth and development [1;2;3]. Previous studies showed microalgae-based biostimulants as a promising, environmentally friendly and sustainable agricultural technique for increasing crop yield and sustainability [4]. Thus, microalgae extracts can be defined as a potential source of nutrition that reduces the need for agricultural land use, decreasing the environmental impact of food production, while improving health [5]. They are also widely used in the aquaculture and poultry industries [6], cosmetics and pharmaceutical industries [6;7;8;9;10], as a source of biofuels, for food and feed supplements [6;11], as biological fertilisers, including macro- and microelements [11]. Microalgae are microscopic single-celled plants that have high rates of growth and photosynthetic efficiency and relatively low natural resource requirements compared to traditionally grown crops [7;12].

Microalgae are photosynthetic microorganisms and one of the oldest forms of life on Earth; however, they remain rather unexplored [13]. Microalgae are a diverse group of unicellular or simple multicellular photosynthetic organisms that can be found in various aquatic environments, such as oceans, freshwater bodies and even in damp soil. They are classified under the group of microorganisms called phytoplankton [14;15]. Microalgae estimate the existence of hundreds of thousands to several million of species. Among these, only approximately 73,000 are currently identified, being a small fraction cultivated at industrial scale for commercial purposes [16]. The

cultivation of microalgae has been studied extensively within the field of biotechnology [11]. Microalgae produce an extensive array of antioxidant molecules, including carotenoids, polyphenols, phycobilins, tocopherols, and essential fatty acids. Carotenoids, such as β -carotene, lutein and astaxanthin [7;8;15;17;18;19], phenolic compounds, chlorophyll a, b pigments [11;20;21] found in certain microalgae species, emerges as a “super antioxidant” with a plethora of health benefits [22]. Microalgae are rich in bioactive compounds such as proteins, carbohydrates, lipids, pigments, vitamins and minerals [8;23]. Owing to their diverse chemical properties, they can act as a nutritional supplement or represent a source of natural food colorants [6]. The antioxidant capacity of microalgae is closely linked to their biochemical composition, which varies across different species, growth conditions, and environmental factors. This variability opens a vast reservoir of potential antioxidant sources, each with unique bioactive profiles and health-promoting properties [18;24;25;26]. Microalgae are able to enhance the nutritional content of conventional food preparations and hence, to affect the health of humans and animals positively [6]. Microalgae extracts can cause plant defense responses by activating enzymes. Cyanobacteria can produce various antimicrobial substances which inhibit growth of various bacteria, fungi, nematodes or even some insects [27]. Although much less studied, microalgal biomass can be used as an organic slow-release fertilizer [11]. The application of these biofertilisers stimulates plant growth and increases crop yields. In addition, the cultivation of persimmon and tomato fruits resulted in improved fruit quality, through higher sugar and carotenoid concentrations in fruits [11;28]. Microalgae-based fertilisers also offer advantages within a larger sustainability framework. In contrast to nutrient-rich waste streams such as manure, microalgal biomass can function as a stable, predictable, transportable and concentrated fertiliser product. This allows it to be introduced in modern greenhouse horticulture. Furthermore, the additional plant growth promoting characteristics of the microalgal biomass demonstrate that nutrient recovery through microalgae cultivation gives an additional value compared with direct application of waste streams on cropland [11;29].

Tomato (*Solanum lycopersicum* L.) is a commercially important crop grown for fresh or processed consumption. Self-compatibility and short life cycles (90–120 days) are appealing factors for agricultural producers [30]. Tomatoes are among the most widely consumed vegetables in the human diet and serve as a source of minerals, vitamins, and antioxidant compounds [31;32;33], while high taste quality and nutritional value [34;35;36] are important for consumers. Tomatoes have gained more popularity in recent years. They contain high amounts of bioactive compounds such as flavonoids, phenolics, anthocyanins, phenolic acids as well as important nutritive compounds such as sugars, essential oils, carotenoids, vitamins, and minerals. Tomato fruits have a distinct flavour, taste together with excellent medicinal value and health care functions [37;38]. Tomato is one of the most consumed vegetables in the world [34;39]. It is grouped as the world’s 2nd essential vegetable crop, grown in almost all regions of the world [34]. Tomatoes are mainly popular as a food because of these factors, also they are important economically, especially in the agricultural sector. The economic benefit of tomatoes is that fruits are used in a wide variety of foods, including sauces, soups, juices, and ketchups [30].

The implementation of innovative nutrient recycling technologies, green fertilizers, and advanced cultivation practices are needed to increase agricultural outputs, improve nutrient use efficiencies and reduce nutrient losses. In modern greenhouses, horticulture is characterized by high crop yields and a stable year-round supply of high-quality fruits and vegetables. This high crop output is often accompanied by the intensive and unsustainable use of biocides, inorganic fertilizers and soilless cultivation techniques [11]. This indicates that nutrients recovered through microalgae cultivation can be recycled as microalgae-based fertilizers to improve the quality and market value of greenhouse crops. Nowadays greenhouse cultivation is the most intensive form of crop production. Greenhouse equipment and covering material provide a controlled microclimate that may be adapted to the needs of the crops, resulting in higher yield, quality and lengthening of the market availability of the products. Greenhouse production requires the use of large amounts of energy, water and agrochemicals, and it usually generates huge quantities of wastes to be disposed

of. Investments, labour and energy costs per unit area are much larger in the greenhouse industry than in any other agricultural sector [40].

The present study aimed to investigate the effect of solution of ethanol extractions of different microalgae species: *Spirulina*, *Dunaliella* and *Chlorella* on the accumulation of biologically active compounds in tomato fruits.

2. Materials and Methods

2.1. Preparation of the microalgae extractions

Microalgae *Spirulina* sp., *Chlorella* sp. and *Dunaliella* sp. were grown autotrophically in 14 L closed-system photo-bioreactors (VariconAqua Solutions Ltd., Great Britain). Water-ethanol extractions with final ethanol concentrations, % v/v 25.0, 25.8 and 23.8 respectively, density (20 °C, g cm⁻³) 0.973, 0.970 and 1.060 respectively, pH 6.9; 6.4 and 5.6 respectively, content of non-volatile substances, % 1.7, 1.3 and 2.7 respectively were obtained from the air-dried (70 °C) microalgae biomass. In the further text, the obtained extraction is considered as 100% for describing the solution concentrations for treatments.

2.2. Plant material and sampling

The experiment was provided in a polycarbonate greenhouse of the Laboratory of Horticulture and Beekeeping of the Institute of Soil and Plant Sciences, Faculty of Agriculture and Food technology, Latvia University of Life Sciences and Technologies. Seeds of tomato cultivar 'Belle' F1 (Enza Zaden) were sown at 1st July of 2023 in plastic cassettes with peat substrate (pH KCl 5.5, producer Laflora Ltd.). At 18th July seedlings were replanted in 1 L pots, but in August 2023, at the age of 35 days, in 25 L pots with the same peat substrate. From seedlings growing till the start of harvesting plants were sprayed weekly, for a total of nine times, with the solution of ethanol extractions of three microalgae species: *Spirulina* sp., *Dunaliella* sp. and *Chlorella* sp. Two concentrations of the extracts (10% and 20% v/v) were compared with sprays with corresponding ethanol solution as a control (2% and 4% v/v) as well as with control spray with drinking water. Nine plants per treatment were used.

During experiment, plant care (irrigation, fertilisation, phytosanitary measures) was provided regularly. Plants were pruned by traditional scheme. Automatic ventilation and additional lighting, provided by high-pressure sodium lamps, were carried out by necessity. Yield was harvested 13 times, once per week, from the end of September (30/09) till the end of December (23/23), at the stage of full ripening (Figure 1).



Figure 1. Maturity stages of tomato: from mature green till full ripening (red), trial photo.

2.3. Determination of vitamin C content

The content of vitamin C was determined titrimetrically using 2,6-dichlorophenol-indophenol [41]. For determination, 2 ± 0.001 g of tomato fruit was quantitatively transferred in 100 mL tubes, added 50 mL of 1% HCl and 5% H_3PO_4 solution (1:1 v/v) and mixed thoroughly. After 30 minutes the solution was filtered through a filter paper. Then 10 mL (V_a) of filtrate was titrated with 0.0005 molar solution of 2,6-dichlorophenol-indophenol (V_{titr}). The content of vitamin C ($mg\ 100\ g^{-1}$) was calculating according to the equation (1):

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

Where:

m – content of vitamin C ;

V_{titr} – volume of 2,6-dichlorophenol-indophenol used for titration, mL;

0.044 v amount of ascorbic acid, which reduce 1 mL 0.0005 M 2,6 dichlorophenolindophenol solution, mg;

V_t – total filtrate volume, mL;

V_a – volume of filtrate (10 mL);

weight – weighed amount of plant material.

2.4. Determination of titratable acidity (TA)

Titratable acidity (TA) was determined titrimetrically [42] with a solution of sodium hydroxide 2 ± 0.001 g of tomato fruit was quantitatively transferred in 100 mL tubes, added 40 mL of distilled water ($0.055\ \mu S\ cm^{-1}$) and mixed. After 30 minutes solutions were centrifuged for 10 min at 5000 rpm. For determination 10 mL of the supernatant was titrated with 0.1 M NaOH in presence of indicator phenolphthalein.

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

Where:

TA – Titratable acidity;

V_{NaOH} – volume of used 0.1 M NaOH;

V_t – total volume (20 mL);

V_s – sample volume (5mL).

Results expressed as g of citric acid 100 g⁻¹ tomato fruit sample fresh matter (FM).

2.5. pH value measurements were determined by standard method LVS ISO 5542:2010 using JENWAY 3520 pH Meter. using a Jenway 3510 pH Meter.

2.6. Determination of lycopene and β -carotene

To determine the concentration of lycopene and β -carotene, samples of 0.5 ± 0.001 g from the tomato fruits were weighed into a tube and 10 mL of tetrahydrofuran (THF) was added [43]. The tubes were sealed and kept at room temperature for 15 min, shaking occasionally, and finally centrifuged for 10 min at 5,000 rpm. The absorbance of the supernatants obtained was determined spectrophotometrically by measuring the absorbance at 663, 645, 505, and 453 nm and then the lycopene and β -carotene contents (mg 100 mL⁻¹) were calculated according to the following equation.

$$C_{lyc} = (0.0458 \times A_{663}) + (0.2044 \times A_{645}) + (0.372 \times A_{505}) - (0.0806 \times A_{453}) \quad (3)$$

$$C_{car} = (0.216 \times A_{663}) - (1.22 \times A_{645}) - (0.304 \times A_{505}) + (0.452 \times A_{453}) \quad (4)$$

Where:

A_{663} , A_{645} , A_{505} and A_{453} —absorption at corresponding wavelength [44].

2.7. Determination of anthocyanins content

Total anthocyanin content was determined by spectrophotometric method according to [45; 46], detected on spectrophotometer Jenway 6705 at wavelength of 540 nm. 20 g of sample was doused with 40 g of ethanol and 1.5 M HCl solution (85:15 by volume) and homogenised for 1 min. Then samples were filtered, and light absorption at 540 nm was detected with a spectrophotometer. Samples were diluted until absorption coefficient was between 0.6 and 0.8. Content mg per 100 g was calculated with the equation (5) [47].

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

Where:

C – total anthocyanin content;

A – absorption coefficient;

v – volume of the extraction (90 mL);

d – dilution;

m – sample weight in g (20);

Measurements were carried out in three replications.

2.8. Determination of total phenols content

The content of total phenols was determined using the spectrophotometric method (detected on spectrophotometer SHIMAZU UV-1800) with its modification. For determination, 1 ± 0.001 g of the plant sample was placed in a graduated test tube, 10 mL of methanol-water-hydrochloric acid solution (79:20:1 v/v/v) was added, shaken for 30 minutes, then separated in a centrifuge (6000 revolutions per minute). The light absorption of the solution is only read at a wavelength of 320 nm

(A₃₂₀). The phenolic content of the plant material (mg GAE g⁻¹) was calculating according to the equation (6):

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

Where:

m – total phenolic content;

A₃₂₀ – absorption determined experimentally at 320 wavelength;

m_{weight} – sample weight, g;

m – phenol content in plant material [48].

2.9. Determination of dry matter and total soluble solids

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

The total soluble solids content (expressed as BRIX degree) was measured with a digital refractometer (A.KRÜSS Optronic Digital Handheld Refractometer DR301-95), calibrated at +20 °C ± 2 °C with distilled water (deviation of measuring instrument face value ± 0.1%) by standard method ISO 2173:2003.

2.10. Determination of fruits mass and taste index

In order to determine the mean weight and the dimensions of tomato fruits, during harvesting, fruits per each sample were counted and weighed using an electric balance with an accuracy of ±0.001 g. Taste index was calculated using soluble solids content and acidity [49; 50].

The taste index (TI) was calculating according to the equation (7):

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

Where:

TSS - total soluble solids, °Brix;

TA- titratable acidity [51].

Connections between fruit mass and taste index in tomato fruits were analysed.

2.11. Statistical analysis

Analyses were performed in three replicates and each one was measured for three repetitions. SPSS 21 (*Statistical Package for Social Sciences*) *Two-way* analyses of variance (*One-Way-ANOVA*) was used. *Scheffe post-hoc* test, *Homogeneity* of variance test were made to determine significance of differences. For mathematical data processing p<0.05; α=0.05 was regarded as statistically significant. Data were expressed as means ± standard deviation (S.D.).

Figure 2 depicts the general scheme of the study.

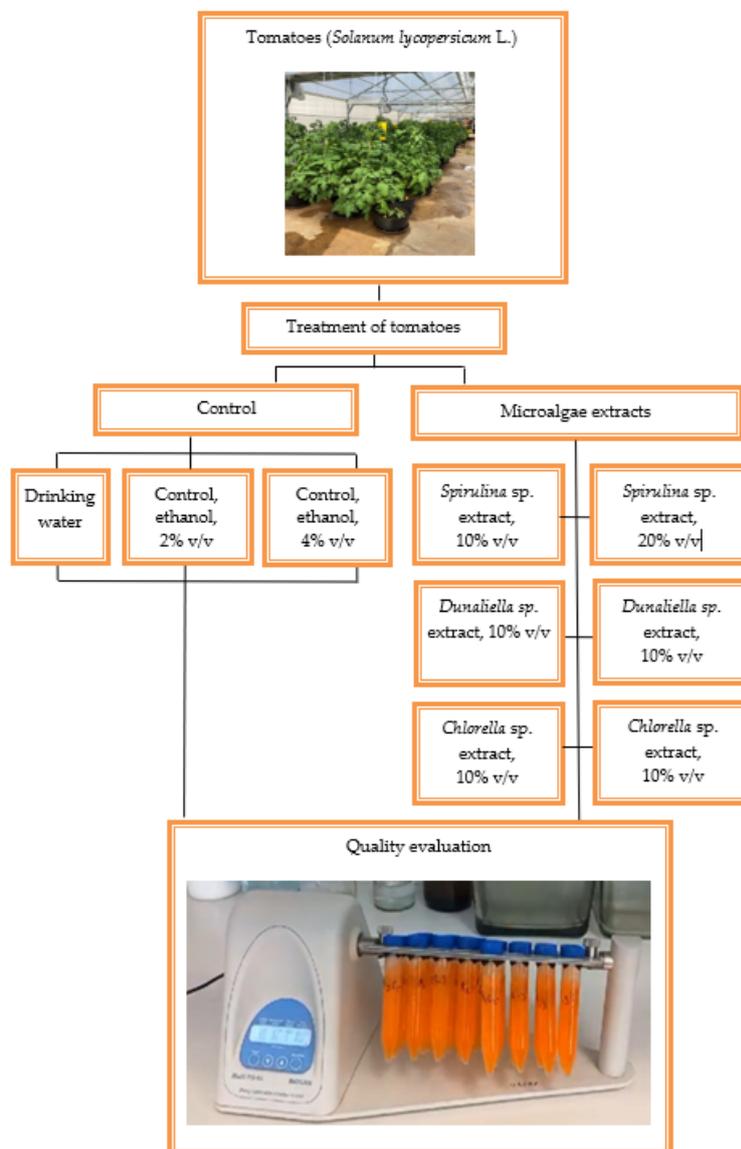


Figure 2. General scheme of study.

3. Results

Vitamin C, determined titrimetrically, is one of the important major phytonutrients in tomatoes. As it is well known, vitamin C is very sensitive to daylight and oxygen. Therefore, the vitamin C extraction and analysis period must be kept as short as possible [52]. Vitamin C content data is represented in Table 1.

Table 1. Quality parameters of analyzed tomato fruits.

Plant material	Vitamin C mg 100 g ⁻¹ (in fresh matter)	Titrateable acidity g 100 g ⁻¹ (in fresh matter)	pH
Drinking water	17.96 ± 0.89 ^a	0.33 ± 0.02 ^a	4.21 ± 0.14 ^a
Control, ethanol, 2% v/v	16.32 ± 0.64 ^a	0.45 ± 0.03 ^a	4.24 ± 0.11 ^{abc}
Control, ethanol, 4% v/v	15.68 ± 0.56 ^a	0.31 ± 0.03 ^a	4.25 ± 0.14 ^{bc}

<i>Spirulina</i> sp. extract, 10% v/v	16.17 ± 0.98 ^a	0.32 ± 0.02 ^a	4.23 ± 0.16 ^{ab}
<i>Spirulina</i> sp. extract, 20% v/v	15.68 ± 0.94 ^a	0.27 ± 0.02 ^a	4.22 ± 0.10 ^a
<i>Dunaliella</i> sp. extract, 10% v/v	18.55 ± 0.74 ^a	0.28 ± 0.01 ^a	4.25 ± 0.15 ^{bc}
<i>Dunaliella</i> sp. extract, 20% v/v	17.21 ± 0.58 ^a	0.25 ± 0.02 ^a	4.25 ± 0.10 ^{bc}
<i>Chlorella</i> sp. extract, 10% v/v	17.47 ± 0.82 ^a	0.32 ± 0.03 ^a	4.30 ± 0.11 ^d
<i>Chlorella</i> sp. extract, 20% v/v	15.47 ± 0.83 ^a	0.28 ± 0.01 ^a	4.27 ± 0.12 ^c

* Titratable acidity expressed as g 100 g⁻¹ of citric acid. * Values, marked with the same letter, are not significantly different (p>0.05; α=0.05).

After mathematical data processing it was proved that the influence of different microalgae species: *Spirulina* sp., *Dunaliella* sp. and *Chlorella* sp. extract concentrations on the Vitamin C content in tomato fruits was not significant (p=0.304; α=0.05). In the present study maximum content of vitamin C 18.55 ± 0.74 mg 100 g⁻¹ (in fresh matter) was found in tomato fruits treated with *Dunaliella*, 10% v/v. In turn with control, ethanol, 4% v/v, *Spirulina*, 20% v/v and *Chlorella*, 20% v/v treated tomato vitamin C content decreased (p=1.000; α=0.05), respectively 15.68 ± 0.56 mg 100 g⁻¹, 15,68 ± 0.94 mg 100 g⁻¹ and 15.47 ± 0.83 mg 100 g⁻¹ (in fresh matter) (Table 1).

The titratable acidity (TA) and pH are two important quality attributes of tomato fruits. Tomatoes are not a low-acid food. The pH of tomatoes is determined primarily by the acid content of the fruit. The acidity of the fruit is also important as a contributor to the flavour of the tomato products [53]. The results of titratable acidity content performed on the tomato fruits samples formulation revealed that titratable acidity varied in the range of 0.25 ± 0.02 g 100 g⁻¹ to 0.45 ± 0.03 g 100 g⁻¹ (Table 1), which indicates acidic environment. The difference between lowest and highest titratable acidity content was not significant (p=0.637; α=0.05). The highest titratable acidity content was found in tomato fruits treated with control, ethanol, 2% v/v (0.45 ± 0.03 g 100 g⁻¹). Little lower results were gained in where tomatoes were treated with *Dunaliella*, 20% v/v, *Spirulina*, 20% v/v respectively 0.25 ± 0.02 g 100 g⁻¹ and 0.27 ± 0.02 g 100 g⁻¹.

The pH value indicates the hydrogen ion concentration which depicts the acidity level [54]. pH value data is represented in Figure 1. pH value of tomato fruits were significantly affected (p<0.05; α=0.05) by different microalgae species levels and their application as well as their interaction. Maximum values of pH (4.30 ± 0.11) were found in tomato fruits treated with *Chlorella*, 10% v/v, while minimum value of pH (4.21 ± 0.14) was noted in the control variant with Drinking water.

Carotenoids are lipophilic pigments that protect plants against photo-oxidative damage [32]. As one of the predominant carotenoids in tomatoes, lycopene is responsible for the colour of tomato fruits [55;56]. It is a bright red carotenoid pigment and phytochemical found in tomatoes and other red fruits [57].

Lycopene content data is represented in Figure 3. Lycopene content of tomato fruits was not significantly affected by different microalgae species and their application's concentration as well as by interaction (p=0.156; α=0.05). Maximum content of lycopene 0.98 ± 0.10 mg 100 g⁻¹ and 0.92 ± 0.06 mg 100 g⁻¹ (in fresh matter) (p=1.000; α=0.05) was observed in tomato fruits treated with control, drinking water and *Chlorella*, 20% v/v, respectively. While minimum content of lycopene (0.60 ± 0.03 mg 100 g⁻¹; 0.66 ± 0.05 mg 100 g⁻¹ and 0.68 ± 0.02 mg 100 g⁻¹ (in fresh matter) was noted, respectively in variants with control, ethanol, 2% v/v; *Dunaliella*, 10% v/v and *Chlorella*, 10% v/v.

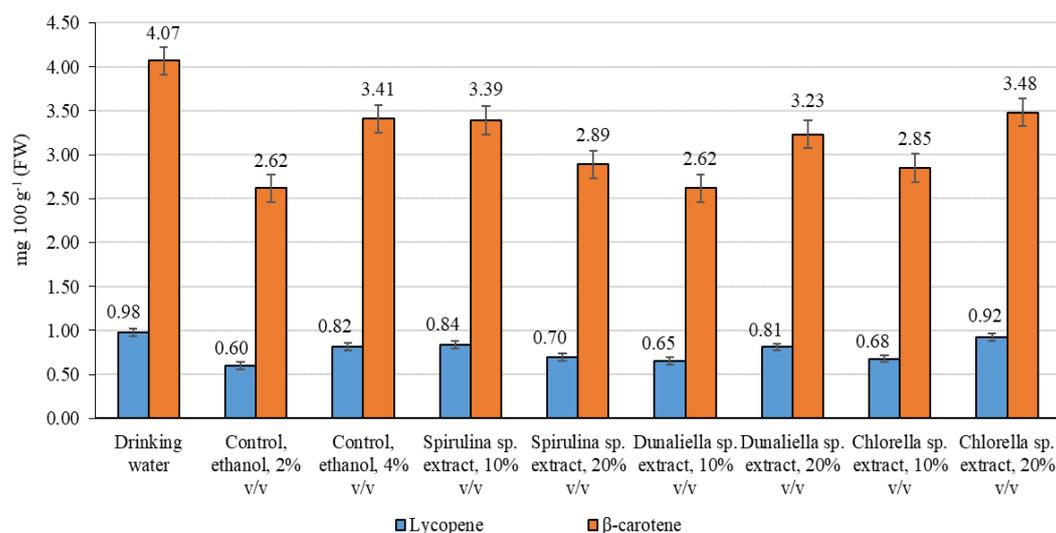


Figure 3. Lycopene and β-carotene content in tomato fruits.

The data in Figure 3 about the content β-carotene of tomato fruit presents no significant effect ($p=0.118$; $\alpha=0.05$) by extractions of different microalgae species and their interaction, similar to lycopene. The maximum content of β-carotene (4.07 ± 0.19 mg 100 g⁻¹ (in fresh matter)) was found in control plants with drinking water. In turn the minimum content of β-carotene (2.62 ± 0.14 mg 100 g⁻¹ and 2.62 ± 0.18 mg 100 g⁻¹ in fresh matter) was noted in tomato fruits under control, ethanol, 2% and *Dunaliella*, 10% v/v.

Polyphenols are secondary metabolites produced by the plant against abiotic and biotic stresses, and are also involved in the processes of plant growth and reproduction [58].

Anthocyanins are blue, red, or purple pigments found in plants, especially flowers, fruits and tubers. These compounds are natural, water-soluble pigments. Anthocyanins appear as a red pigment in an acidic environment, and as a blue pigment in alkaline conditions. Significant differences are not observed for anthocyanins' content in the tomato fruits between different microalgae species: *Spirulina*, *Dunaliella* and *Chlorella* as well as control samples ($p=0.708$; $\alpha=0.05$) (Table 2). On average for all tomato samples, content of anthocyanin was stated between 0.07 and 0.42 mg 100 g⁻¹ (in fresh matter). However, it should be noted that the content was higher ($p>0.05$; $\alpha=0.05$) in tomato samples under control with drinking water, respectively 0.42 ± 0.04 mg 100 g⁻¹ (in fresh matter). And the lowest anthocyanin content was shown by control, ethanol, 4% v/v (0.09 ± 0.01 mg 100 g⁻¹) ($p>0.05$; $\alpha=0.05$).

Table 2. Anthocyanins and total phenols in tomato fruits.

Plant material	Anthocyanins mg 100 g ⁻¹ (in fresh matter)	Total phenols mg 100 g ⁻¹ (in fresh matter)
Drinking water	0.42 ± 0.04^a	166.93 ± 2.01^c
Control, ethanol, 2% v/v	0.09 ± 0.01^a	137.59 ± 1.34^a
Control, ethanol, 4% v/v	0.07 ± 0.01^a	142.21 ± 1.53^{ab}
<i>Spirulina</i> sp. extract, 10% v/v	0.12 ± 0.02^a	144.21 ± 1.87^{ab}
<i>Spirulina</i> sp. extract, 20% v/v	0.11 ± 0.01^a	142.23 ± 1.33^{ab}

<i>Dunaliella</i> sp. extract, 10% v/v	0.14 ± 0.02 ^a	142.22 ± 2.02 ^{ab}
<i>Dunaliella</i> sp. extract, 20% v/v	0.09 ± 0.01 ^a	143.33 ± 1.22 ^{ab}
<i>Chlorella</i> sp. extract, 10% v/v	0.11 ± 0.01 ^a	145.96 ± 2.04 ^{ab}
<i>Chlorella</i> sp. extract, 20% v/v	0.12 ± 0.01 ^a	145.49 ± 2.25 ^{ab}

*Values, marked with the same letter, are not significantly different ($p > 0.05$; $\alpha = 0.05$).

Data about the total phenol content in tomato fruits is represented in Table 2. Total phenol content was significantly affected by different microalgae species and their interaction ($p < 0.05$; $\alpha = 0.05$). Maximum total phenol content (166.93 ± 2.01) was found in tomato fruits under control with drinking water ($p = 0.04$; $\alpha = 0.05$). In turn minimum total phenol content (137.59 ± 1.34) was noted under control, ethanol, 2% v/v.

The components in the fruit consist of water-soluble components, such as glucose, sucrose, fructose, and water soluble proteins (pectin) [54]. Soluble solids is the content of total soluble sugars, determined refractometrically in °Brix units. Soluble solid is one of the most important quality factors of fruits and vegetables. On the other hand, it is known that the content of soluble solids changes during the ripening of tomato fruits. Figure 4 depicts the content of dry matter (%) and total soluble solids (°Brix) in tomato fruits which are treated with different microalgae extracts. Our study results showed that the content of total soluble solids of tomato fruits were significantly affected by different microalgae species and extracts' application as well as their interaction. Maximum content of total soluble solids were 4.4 ± 0.18 °Brix (control, drinking water) and 4.29 ± 0.05 °Brix (*Dunaliella* sp. extract, 20% v/v) while minimum content of total soluble solids was 3.93 ± 0.12 (*Chlorella* sp. extract, 20% v/v).

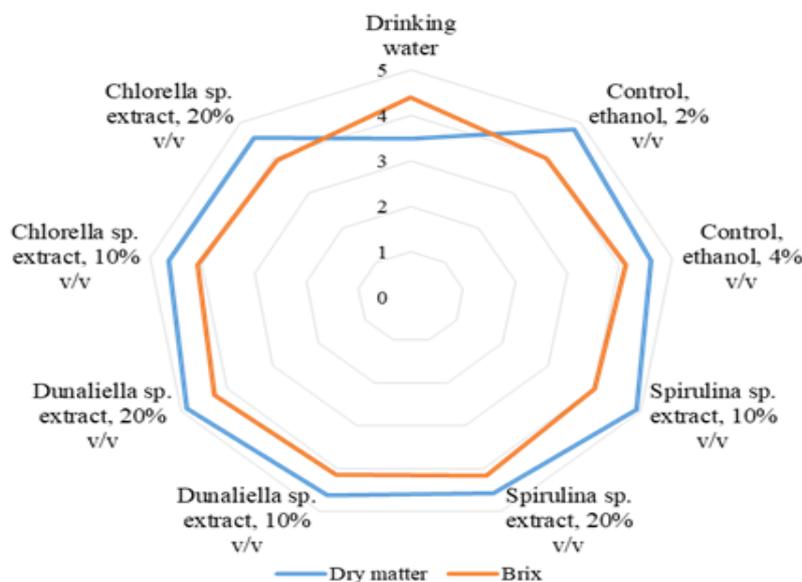


Figure 4. Content of dry matter (%) and total soluble solids (°Brix) in tomato fruits.

Content of dry matter of the tomato fruit samples presented no significant differences between different microalgae species ($p = 0.785$; $\alpha = 0.05$) (Figure 4). The content of dry matter performed on the fresh tomato fruits samples formulation revealed that the content of dry matter ranged from 3.49% to 4.91%.

By fruit mass (Table 3), maximal result (325.76 g) was observed for the control variant with ethanol, 4% v/v, but the taste index was 0.34 only. Minimal fruit mass (34.7 g) was observed for the variant with *Chlorella*, 10% v/v, taste index 0.33 respectively. The higher taste index was observed for both variants with *Spirulina* sp. extract: 0.57 under 10% v/v and 0.54 under 20% v/v (Table 3). For

these variants, average fruit mass was 101.66 g and 116.15 g respectively. The minimal taste index was observed for variants with drinking water (0.31), average fruit mass was 92.49 g for this variant.

Table 3. Connections between fruits mass and taste index in tomato fruits.

Plant material	Tomato fruit mass, g				Taste index
	Average p=0.673	Minimum	Maximum	S.D.	
Drinking water	92.49 ^a	43.05	200.01	44.81	0.31
Control, ethanol, 2% v/v	95.11 ^a	45.08	170.00	40.47	0.38
Control, ethanol, 4% v/v	115.21 ^a	37.89	325.76	67.09	0.34
<i>Spirulina</i> sp. extract, 10% v/v	101.66 ^a	39.25	185.73	46.71	0.57
<i>Spirulina</i> sp. extract, 20% v/v	116.15 ^a	40.34	213.28	47.77	0.54
<i>Dunaliella</i> sp. extract, 10% v/v	129.33 ^a	55.71	273.75	68.08	0.37
<i>Dunaliella</i> sp. extract, 20% v/v	102.52 ^a	39.45	218.00	59.36	0.39
<i>Chlorella</i> sp. extract, 10% v/v	94.85 ^a	34.7	188.75	48.63	0.33
<i>Chlorella</i> sp. extract, 20% v/v	104.78 ^a	55.27	195.21	42.02	0.33

*Values, marked with the same letter, are not significantly different ($p > 0.05$; $\alpha = 0.05$). S.D. standard deviation.

In averages, the highest result (129.33) in fruit mass was observed for the variant with *Dunaliella*, 10% v/v, while the closest average fruit mass (92.49 g) was noted under control with drinking water. The difference between average tomato fruit mass, g (Table 3) was not statistically significant ($p = 0.673$; $\alpha = 0.05$). Excluding variants with *Spirulina* sp., influence of the type and concentration of algae extract on the taste index was not observed.

4. Discussion

In present research, the content of vitamin C in tomatoes varied from 15.47 till 17.96 mg 100 g⁻¹. Scientists from Romania reported that the content of vitamin C in tomatoes was 15.5 – 20.7 mg 100 g⁻¹ [59]. From the Slovak researchers' results [60], it is possible to conclude that the average content of vitamin C in tomato fruits was in the range 17–21 mg 100 g⁻¹ (in fresh matter). Similar values are shown by a study of Latvian scientists where the vitamin C content 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 [61; 62; 63] (in fresh matter).

By scientists from Portugal [59], more mature tomatoes have higher pH. On the other hand, the acid content has an increasing tendency during ripening to the breaker stage and then results decrease. This can describe the pH results 4.21–4.30 in current study.

In scientific articles it is described, that the composition of carotenoids can vary quantitatively and qualitatively, and it can be affected by storage conditions and duration, variety [64; 65] as well as climate, season, geographic location, maturity phase [66]. Similar results as in our research about

the lycopene content in tomatoes were found in the scientific literature analysed in Latvia, where data varied 0.07 – 27.11 mg 100 g⁻¹ [42; 67].

Also in the case of β -carotene, similar results were found in the scientific literature about tomatoes analysed in Latvia: 0.04 – 11.73 mg 100 g⁻¹ [42; 67]. 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 [68;69]. The antioxidant potential of lycopene is higher than of β -carotene [70;71]. Researchers [11] accepted that different microalgae species extract influence higher or lower concentration of carotenoids in tomato fruits.

Scientists from Latvia [42] reported that the comparison of biochemical compounds is often difficult due to the great influence of agronomic factors such as varieties, growing conditions and harvesting time. Furthermore, this content increases from the mature green stage to the red stage [72;73]. Soluble solid affects the sweetness of vegetables [74] and length of storage period, as well as quality characteristics [75].

Scientific literature mentions that the dry matter content in tomatoes was between 5.42 to 8.25% [41]. The content of soluble solids in tomatoes grown by scientists was determined similarly: from 3.8 to 10.2 °Brix [42] and 1.11 to 7.2 °Brix [59]. On the other hand, the taste index of Latvian tomatoes described in the articles varied between 0.95 and 1.38 [42; 67], 0.95 to 1.26 [41]. Our data can be described that tomatoes were grown in autumn-winter, not in summer cycle. But in other article it was similar data, where the taste index was from 0.34 to 0.53 [59],

The obtained results regarding tomato fruit mass are similar with other results [41], but [76;77] reported fruit mass between 66 to 158 g and 107 to 185 g, respectively, which are lower than our study results.

Latvian scientists study [41] reported that customers often pay attention not only to the colour of tomato fruits, but also to the size. When comparing the results of fruits mass and taste index, it is observed that higher taste index is for smaller or bigger tomatoes. In turn an interesting conclusion has been discovered by researchers from China [78], who conclude in their study that when tomato fruit size is reduced due to reduced irrigation or high salinity, applying additional stress may reduce fruit susceptibility. Therefore, it is necessary to consider a reasonable water regime in the practical management of tomatoes grown in saline soil regions to ensure high-quality tomato production.

Scientists [2] have proven in their research that microalgae-based bio-stimulants stimulates cucumber and tomato seeds germination, which could possibly increase fruit mass as well, thereby [11;79] raising the biomass, stimulated plant growth and increased crop yields and quality. Furthermore, scientists [11] reported that nutrients recovered through microalgae cultivation can be recycled as microalgae-based fertilisers to improve the quality and market value of high-value fruits such as tomatoes.

5. Conclusions

The obtained results showed that maximum content of vitamin C 18.55 ± 0.74 mg 100 g⁻¹ (in fresh matter) was found in tomato fruits treated with *Dunaliella* sp. extract, 10% v/v. The highest titratable acidity content was found in tomato fruits treated with control, ethanol, 2% v/v (0.45 ± 0.03). Maximal value of pH 4.30 ± 0.11 was found in tomato fruits treated with *Chlorella* sp. extract, 10% v/v. Maximal content of β -carotene (4.07 ± 0.19) was found in control plants with drinking water. Maximal content of lycopene 0.98 ± 0.10 mg 100 g⁻¹ and 0.92 ± 0.06 mg 100 g⁻¹ (in fresh matter) ($p=1.000$; $\alpha=0.05$) was observed in tomato fruits treated with drinking water and *Chlorella* sp. extract, 20% v/v, respectively. The anthocyanin content was higher ($p>0.05$; $\alpha=0.05$) in tomato samples under control variant with drinking water: 0.42 ± 0.04 mg 100 g⁻¹ (in fresh matter). Maximum total phenol content 166.93 ± 2.01 was found in tomato fruits treated with drinking water ($p=0.04$; $\alpha=0.05$). Maximum content of total soluble solids were 4.4 ± 0.18 °Brix under control with drinking water and 4.29 ± 0.05 °Brix under treatment with *Dunaliella* sp. extract, 20% v/v. The content of dry matter performed on the fresh tomato fruits samples formulation revealed that the content of dry matter ranged from 3.49% to 4.91%. By the taste index, higher results were under *Spirulina* sp. extract, 10% v/v and *Spirulina* sp. extract, 20% v/v, respectively 0.57 and 0.54.

Our study showed the influence of microalgae-based bio-stimulants *Spirulina* sp., *Dunaliella* sp. and *Chlorella* sp. on quality parameters of tomatoes are different. As statistically significant influence was observed only for pH values, total phenol content and total soluble solids, results can be described with the genetical stability of the cultivar. No negative effects of any tested extracts on biologically active compounds in tomatoes were observed.

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