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Stimulation of plant secondary metabolites synthesis in soilless cultivated strawberries (*Fragaria* × *ananassa* Duchesne) using zinc-alginate microparticles

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Abstract: An innovative approach to stimulate the production of plant secondary metabolites (PSM) is the use of encapsulated bioactive agents. Zinc-alginate microparticles [microspheres (ALG/Zn) and microcapsules (CS/ALG/Zn)] were applied near the root zone of soilless cultivated strawberries (*Fragaria* × *ananassa* Duchesne) to ensure a constant supply of an essential micronutrient zinc ions throughout the whole period of maturation. Both, ALG/Zn and CS/ALG/Zn had a positive effect on PSM (total polyphenols, total flavonoids, total anthocyanins, and flavan-3-ols) synthesis and increased antioxidant activity in two strawberry varieties (Albion and San Andreas). Treatments with Zn-alginate microparticles resulted in no visible damages on the plant and fruits. A two-year study revealed an increase of ~34% in total polyphenolic compounds and ~23% in total flavonoids in the San Andreas variety. The highest increase in antioxidant activity [ABTS (~37%) and DPPH (~38%)] was observed in the San Andreas variety treated with CS/ALG/Zn. Respectively high increase in anthocyanins was observed in both varieties (~54% and ~61%, respectively) treated with CS/ALG/Zn. Treatments with zinc-alginate microparticles resulted in a successful stimulation of PSM synthesis and increased strawberries' nutritive value. Encapsulation of zinc ions proved to be simple, sustainable, and environmentally favorable to produce strawberries fortified with important bioactive compounds.

Key words: Zinc-alginate microparticles, encapsulation, soilless cultivation, strawberries, plant secondary metabolites, antioxidants

1. Introduction

Strawberries (*Fragaria* × *ananassa* Duchesne), a member of the Rosaceae family, are very popular fruits in either fresh or processed forms due to their flavor and deliciousness. Strawberries are produced all over the world, mostly in open fields as well as in greenhouses. Since it has tremendous commercial and economic effects, it can be considered as the most researched berry from a dietary, genomic, or agronomic point of view (Giamperi et al, 2012).

There has been a strong link between the intake of strawberries and the health benefits from the reported anticancer, antiinflammatory, and antineurodegenerative properties of strawberry fruits as well as delay aging and prevent the occurrence of age-related diseases (Giamperi et al, 2017). These health-promoting effects are, at least partially, the result of the presence of plant secondary metabolites (PSM). PSM are various chemical (bioactive) compounds synthesized by the plant cell through metabolic pathways derived from the primary metabolic pathways. Polyphenols are plant secondary metabolites that are also often characterized by high antioxidant activity. The presence of these components influences the quality, nutritional value, and sensory characteristics of strawberries. Their presence and quantity were used as markers for the differentiation between cultivars and for the definition of different methods of processing (Karlund et al, 2015). Depending on the cultivar, growing conditions, and methods, as well as the degree of maturity, strawberry fruit can differ in the composition of polyphenols. The main group of polyphenols in strawberry fruit are anthocyanins, the major of which are pelargonidin-3-glucoside and cyanidin-3-glucoside. Their quantification is important to breeders because it enables them to determine the nutritional value of the mature fruit. The second most abundant polyphenols are flavan-3-ols, namely proanthocyanidins and their monomers. Flavonols are another significant group of compounds found in strawberries, with the dominant members being quercetin and kaempferol glucosides, and glucuronides (Michealska et al, 2017).

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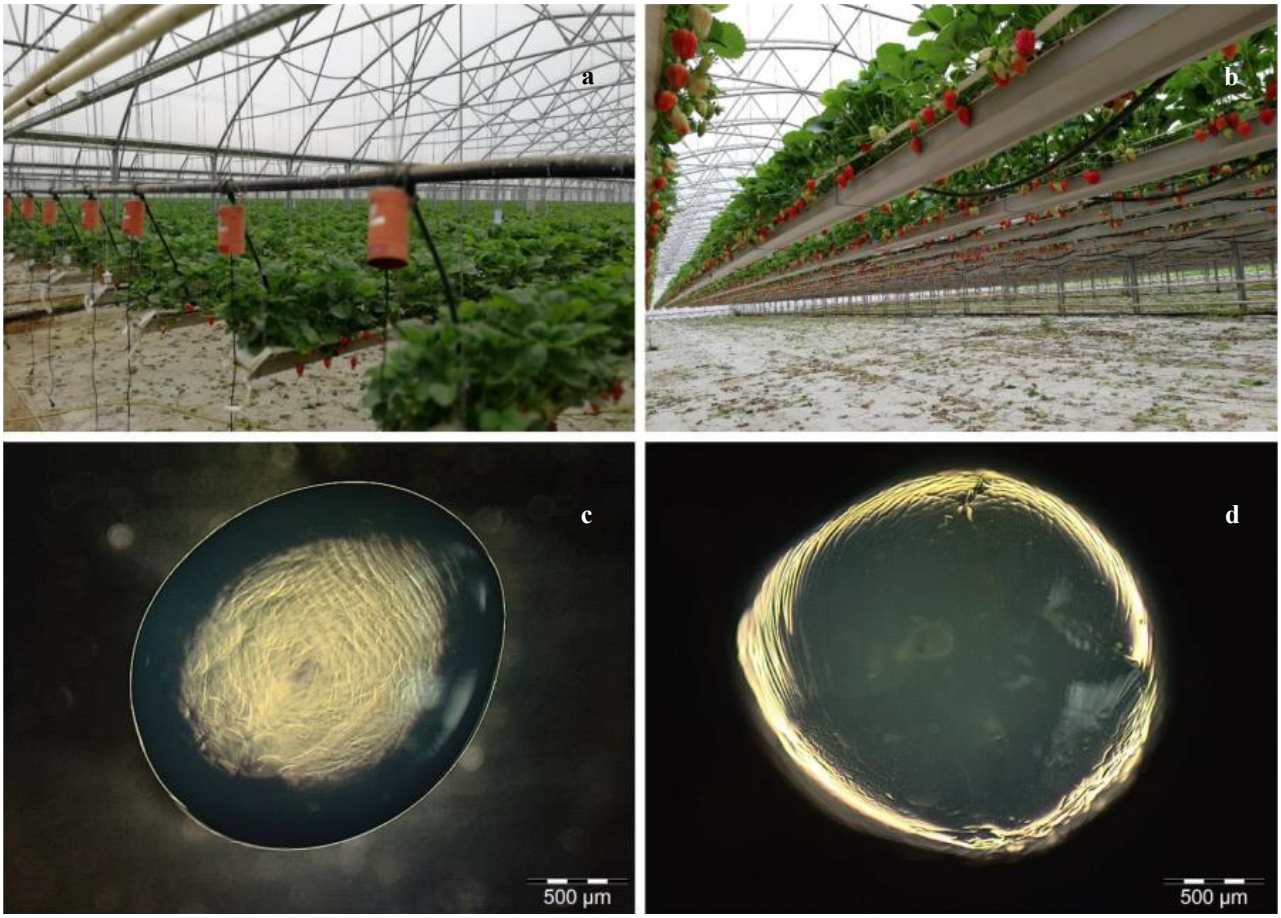

Figure. Place of the experiment (a and b), soilless cultivation of strawberries (*Fragaria* × *ananassa* Duch.). Zn-alginate microsphere (c) and Zn-alginate microcapsule (d) under the phase-contrast light microscope. Scale bars are indicated.

There is an increasing demand for strawberries in the summer months which makes producers interested in producing strawberry fruits of higher quality (Duralija, 2006). Environmental and agronomic factors highly influence berry nutritional quality. The nutritional quality of berries, in terms of bioactive compounds with healthy effects on the final consumer, is used to better characterize the fruit quality. Nutritional quality is of primary importance and goal for the breeders since not only this benefits consumer but also protects strawberries against negative environmental factors (Di Vittori et al., 2019).

Since Zn is part of many enzymes and other cell proteins, it is an essential metal for normal plant growth and development. It is also required for the synthesis of tryptophan, a precursor of indole-3-acetic acid which acts as a growth-promoting substance. Zinc deficiency is the most widespread micronutrient deficiency problem in crop plants. It can affect plants by stunting their growth and decreasing the number of tillers and smaller leaves. It can induce chlorotic spots on leaves, increase crop maturity period, and result in an inferior quality of harvested products. Zinc deficiency is easily distinguished by the green halo that develops along the serrated margins of young immature leaf blades. Yellowing and green veining are also common in Zn-deficient strawberry plants. (Trejo-Tellez and Gomez-Merin, 2014).

Foliar application of zinc sulfate increases fruit quality and yield of strawberries. Specifically, preharvest application influences plant dry weight, number of runners, leaf area, length of roots, and number of flowers. It also affects the length of the flowering period, primary and secondary fruits, as well as the number of achenes (Kazemi, 2014).Young leaves, flowers, and fruits can be affected using zinc sulfate as a foliar spray, but this method is not sustainable and economic due to the overuse and spillage of the material. Also, in terms of the occurrence of leaf burning associated with toxic saline symptoms of more concentrated fertilizer solutions, a different approach to new fertilizer methods is required (Farid et al., 2020).

Encapsulation of zinc ions in biopolymeric microparticles via ionic gelation method and its application to the strawberry plants present an innovative approach to

stimulate the production of plant secondary metabolites. Bipolymers are great materials for encapsulation due to their safety for the environment, better stability and stabilization of the encapsulated material, controlled release at a specific target, longer retention time, cost-effectiveness, and easier physical handling (Jurić et al., 2020).

In this research, for the first time, zinc-alginate microparticles were used to promote the synthesis of polyphenols in soilless grown strawberries (*Fragaria* × *ananassa* Duch.) to yield highly nutritious strawberry fruits.

2. Materials and methods

2.1. Preparation and application of microparticles (microspheres and microcapsules)

Microspheres were prepared by the ionic gelation method, and microcapsules by chitosan/alginate polyelectrolyte complexation on the surface of microspheres as was previously described (Vinceković et al., 2016, Vinceković et al., 2017a, 2017b). Büchi Encapsulator B-390 was used to produce the jet chainand the procedure is detailed described in the literature (Jurić et al., 2019a, 2019b). The carrier used for the encapsulation process was 1.5% sodium alginate (w/v) and the gelling cation (Zn^{2+}) donor was 0.5 mol dm^{-3} $ZnSO_4 \times 7H_2O$ solution. The used nozzle on the encapsulator was 1000 µm size and the vibration frequency was set to 40 Hz (Amplitude 3) at the gas (nitrogen) pressure of 0.2 bar resulting in a flow rate of 30–40 mL min^{-1}. Zn-alginate microspheres were prepared by dripping 1.5% sodium alginate in Zn^{2+} containing a bath. The resulting particles were left to stir in the gelling donor bath for an hour to harden and saturate with Zn^{2+} ions. Afterward, microspheres were washed with distilled water three times. Part of the microspheres was further used to produce microcapsules. Briefly, microspheres were put in chitosan [0.5% high molecular weight chitosan (w/v) in 1% CH_3COOH (v/v)] solution for an additional coating to obtain microcapsules. After 30 min, microcapsules were washed three times with distilled water and stored until the day after (treatment day) at 4 °C in the dark. Prepared microparticles (microspheres and microcapsules) were used for the treatment of two strawberry varieties (Albion and San Andreas) in soilless cultivation (Figure). Two treatments (microspheres with Zn^{2+} (ALG/Zn) and microcapsules with Zn^{2+}, coated with chitosan (CS/ALG/Zn) were carried out by applying 4 grams of microparticles near the root of the plant (8.47 mg of Zn^{2+} g^{-1} of microparticles) with control as untreated plants. The research was conducted for two consecutive years, where new plants (and within new blocks) were treated each year.

Trials were set up on strawberry farm Jagodar-HB Ltd situated near Zagreb, Croatia (Petrovina Turopoljska, latitude N45°41'14.67" and longitude E16°01'5.84") and grown in bags with coconut coir. Two day-neutral strawberry cultivars Albion and San Andreas were planted in the density of 10 plants m^{-2} as green container plants in November of the year before harvest. Standard nutrition management was applied to all plants, pH value in the root zone was between 5.5 and 6.5 and EC of drainage solution was under 2 dS m^{-1} during growing and harvest. A total of 180 plants were used in this research, 15 plants in 4 repetitions (total of 60 plants per treatment) were treated in random block design with untreated plants as control. Equal treatments with microparticles were carried out for two consecutive years in spring (May 5th, 2018 and May 8th, 2019). Fruits were harvested about two months later in summer (July 9th, 2018 and July 8th, 2019) and transported to the Laboratories of the Faculty of Agriculture at the University of Zagreb for analysis.

2.2. Determination of zinc ions content in microparticles

Microparticles were dissolved in buffer solution containing 0.2 mol dm^{-3} $NaHCO_3$ and 0.06 mol dm^{-3} $Na_3C_6H_5O_7 \times 2H_2O$ (pH 8.28).The obtained solution was subjected to the inductively coupled plasma-optical emission spectroscopy (VarianVista Pro-axial; Varian Inc., Varian Australia Pty Ltd., Mulgrave, VIC, Australia)for the determination of Zn^{2+} ions.

2.3. Optical microscopy and particles size determination

The average diameter of wet microparticles was determined by optical microscopy using Olympus Soft Imaging Solutions GmbH, version E_LCmicro_09Okt2009. Sixty microparticles were randomly selected from each batch produced in triplicate, to determine the particle size distribution (Figure).

2.4. Preparation of fruit juice for analysis

Immediately after the harvest, fresh strawberry fruits were crushed and homogenized using a laboratory homogenizer [FOSS homogenizer 2094 (Hillerød, Denmark)]. Suspensions were centrifuged (9000 rpm, 20 min) and supernatants were filtered through the Whatman No.4 filter paper and used for further analysis. The juice was diluted when necessary.

2.5. Determination of total polyphenolic compounds (TPC)

For the determination of TPC, a modified Singelton et al. (1999) method was used. A volume of 100 µL of strawberry juice was mixed with 7.9 mL of distilled water and 0.5 mL Folin Ciocalteu's reagent (diluted with distilled water in a 1:2 ratio). The suspension was mixed with 1.5 mL of 20% Na_2CO_3 (w/v) and vortexed. After 2 h absorbance was measured at 765 nm, and the data were expressed as gallic acid equivalents per L of juice (mg GAE L^{-1}).

2.6. Determination of total flavonoids content (TFC)

TFC was determined using a modified spectrophotometric method of Ivanova et al. (2010). One mL of strawberry

juice was added to a 10 mL volumetric flask containing 4 mL of distilled water. The volume of 300 µL of $NaNO_2$ (0.5 g L^{-1}) solution was added to the suspension and after 5 min, 300 µL of $AlCl_3$ (1 g L^{-1}), respectively. After 6 min, 2 mL of NaOH (1 mol L^{-1}) was added to the mixture. The final volume was set to 10 mL with the addition of distilled water. Absorbance was measured at 360 nm and calculated as mg quercetin equivalents per L of juice (mg QE/L).

2.7. Determination of total anthocyanins (TA)

TA was determined using a modified method with 1% (v/v) hydrochloric acid in 70% EtOH solution (Fuleki and Francis, 1968). Juice samples were diluted, added to the extraction solution and absorbance was measured at 525 nm. Results were calculated as per the equation:

$$Total\ anthocyanins = \frac{A_{525}\ x\ Mr(malvidin-3-glucoside)\ x\ 1000}{\varepsilon\ x\ 5}$$

where ε is the molar extinction coefficient of malvidin-3-glucoside. Results are expressed as mg malvidin-3-glucoside equivalents per L of juice (mg M3GE L^{-1}).

2.8. Determination of flavan-3-ols

Flavan-3-ols were determined using the *p*-(dimethylamino) cinnamaldehyde (*p*-DMACA) method. Briefly, 1 mL of diluted juice sample was added to a 10 mL volumetric flask. Three drops of glycerol were added along with freshly prepared 5 mL *p*-DMACA reagent [1%(w/v) in a cold mixture of methanol and HCl (4:1)]. Volume was set to 10 mL with the addition of methanol and after 7 min absorbance was measured at 640 nm (Ivanova et al., 2010). The contents of flavan-3-ols are expressed as mg (–)-epicatechin equivalents per L of juice (EE mg-L^{-1}).

2.9. Determination of antioxidant activity (AA)

The AA of strawberry juice was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) reagents, according to the well-known procedures of Brand-Williams et al. (1995) and Re et al. (1999), respectively. The data obtained are expressed as µmol Trolox equivalents per L of juice (µmol TE L^{-1}).

2.10. Statistical analysis

Statistical analysis was performed using Excel XLSTAT and IBM SPSS Statistics v. 22 software. One-way ANOVA was performed, and significant differences were observed based on the posthoc Tukey HSD test ($p < 0.05$). The relative change (%) was calculated using the formula:

$$Rc\ (\%) = \frac{treatment\ -\ control}{control} x\ 100$$

Results are expressed as mean values with corresponding standard deviation (AVG ± SD).

3. Results and discussion

In this two-year research, we report how zinc-alginate microparticles (ALG/Zn and CS/ALG/Zn) affect strawberry fruits in terms of quality, as per the amount of biologically active compounds and total antioxidant activity.

It is well known that plant secondary metabolites (PSM) have importance in various functions from plant pigmentation, growth, and reproduction to resistance to pathogens. plant secondary metabolites are the result of the adaptive features of plants that have undergone natural selection during evolution (Jurić et al., 2020). They can be utilized in a wide array of industrial applications, from the production of functional foods to medicinal and cosmetic products. In conclusion, PSM is a valuable compound important for numerous reasons.

Herein we report the application of microparticles containing an active ingredient, zinc ions (Zn^{2+}) on strawberries (two varieties) cultivated in soilless. Zn^{2+} is known to control mechanisms of generation and detoxification of oxygen- derived radicals and activation of various antioxidant enzymes. It has an essential role either as a metal segment of enzymes or as a functional, structural, or controlling cofactor of a large number of enzymes (Cakmak and Marschner, 1993; Yu et al., 1998; Wang and Jin, 2007; Tavallali et al., 2017). The source of nutrients plays a crucial role in the absorption of zinc (Cakmak, 2000). In general, foliar fertilizers (i.e. $ZnSO_4$ or EDTA-Zn chelate) are applied as solutions to the leaves or ground and the application is performed by spraying the solution. This has proven to be not effective enough due to poor absorption and high losses during the process of application (Fageria et al., 2002; Doolette et al., 2018).

An innovative approach to stimulate the production of PSM is the use of an encapsulation method for targeted and sustained delivery of chemical agents (Vinceković et al., 2016; Jurić et al., 2020). Encapsulation of zinc ions ensures that it remains in a form that is easily uptaken by the plant (Jurić et al., 2020). Also, the application of Zn-alginate microparticles near the root of the plant keeps the constant supply of these nutrients throughout the whole period of maturation. Plant roots uptake zinc mainly as a divalent cation (Zn^{2+}) and this depends on the ligand secreted by plant roots and two physiological ways that are involved in the uptake of Zn^{2+}. The first involves efflux of reductants, organic acids, and H^+ ions, which enhance the solubility of Zn-complexes and release Zn^{2+} ions for absorption by root epidermal cells. Secondly, it involves the efflux of phytosiderophores (phytometallophores) which form stable complexes with Zn and their subsequent influx into root epidermal cells (Gupta et al., 2016).

It has been previously documented that zinc application influences the composition of various plant parts, such as essential oils, total polyphenols, and antioxidant activity (Grotz and Guerinot, 2006; Misra and Sharma, 1991; El- Sawi and Mohamed, 2002; Derakhshani et al., 2011).

The zinc-exposed plants had a high expression of lignin biosynthesis genes (Van de Mortel et al., 2006) which reveals the involvement of this metal in the shikimic acid pathway which consequently affects the polyphenol content in the fruit. From the results presented in Table 1, we can conclude that both ALG/Zn and CS/ALG/Zn significantly encouraged the synthesis of total polyphenols in strawberries. In both varieties, we can observe an increase of up to 34.26% for San Andreas and 26.08% for the Albion variety, relative to the respective control samples. Furthermore, regardless of the varieties, a somewhat lower increase in TPC was observed with CS/ALG/Zn treatment, due to the presence of chitosan coat on the surface of microcapsules which causes retention and slower release of the active agent from the core of the microcapsule (Vinceković et al., 2016). The TPC in strawberries varies from 1086.44 to 1907.87 mg GAE L^{-1} juice depending on the treatment and variety. To compare obtained values with the existing literature, Diaz-Garcia et al. (2013) reported a TPC of 1406.12 mg GAE L^{-1} of strawberry juice which is in the range reported here. Šamec et al. (2016) determined a range of TPC from 1711.6 to 1889.9 mg GAE L^{-1} in Albion cultivar grown near Zagreb, but the fruits were harvested out of season (autumn). Song et al. (2015) performed foliar spraying of zinc sulfate which significantly influenced the expression of phenolics biosynthetic pathway genes throughout berry (*Vitis vinifera* L.) development. Obtained results of expression analysis supported the promotion of Zn treatments on phenolics accumulation. Various genes are responsible to stimulate enzyme syntheses which are involved in phenols and flavonoids synthesis and this can significantly alleviate with Zn treatments. This suggests that the promotion of phenolic compound biosynthesis in zinc-treated berries (*Vitis vinifera* L.) could be caused by the higher expression of genes in phenolics biosynthetic pathways at veraison (Song et al., 2015).

When discussing the results on total flavonoids, results are in correlation with TPC (r = 0.75) (Table 2). The highest total polyphenolic content increase was observed in the San Andreas variety. This may be ascribed to the pH lowering of the surrounding media due to the presence of slightly acidic CS/ALG/Zn. From the literature and our observations, the production of TFC is significantly influenced by the lower pH which enables higher production rates and accumulation of flavonoids in plants (Radić et al., 2016; Jurić et al., 2020) thus the results from CS/ALG/Zn treatments appear to be higher. The influence of treatments on the Albion variety was somewhat smaller, especially in the second year. The inconsistency of the results between the two seasons may be attributed to the uneven conditions in the surrounding media. In addition

Table 1. Total polyphenolic content (TPC) in nontreated (control) and with microparticles (ALG/Zn or CS/ALG/Zn) treated strawberries (two varieties) – two-year soilless cultivation. Results are expressed per volume of strawberry juice.

Variety	Treatment	TPC (mg GAE L^{-1} juice)	*Rc (%)
Year 1			
Albion	Control	1412.37 ± 130.47^{a}	
	ALG/Zn	1777.19 ± 186.76^{b}	+25.82
	CS/ALG/Zn	1672.56 ± 87.62^{b}	+18.42
San Andreas	Control	1595.70 ± 164.83^{a}	
	ALG/Zn	1907.87 ± 162.44^{b}	+19.56
	CS/ALG/Zn	1853.11 ± 36.00^{b}	+16.13
Year 2			
Albion	Control	1443.85 ± 42.38^{a}	
	ALG/Zn	1614.22 ± 57.74^{b}	+11.80
	CS/ALG/Zn	1488.30 ± 17.74^{a}	+3.08
San Andreas	Control	1086.44 ± 189.95^{a}	
	ALG/Zn	1458.67 ± 71.66^{b}	+34.26
	CS/ALG/Zn	1369.78 ± 23.57^{ab}	+26.08

*Represents a relative change (%) compared to the respective control. Values superscripted with the same letter are not significantly different according to the posthoc t-tests with Bonferroni correction ($p < 0.05$).

Table 2. Total flavonoids content (TFC) in nontreated (control) and with microparticles (ALG/Zn or CS/ALG/Zn) treated strawberries (two varieties) – two-year soilless cultivation. Results are expressed per volume of strawberry juice.

Variety	Treatment	TFC (mg QE L^{-1} juice)	*Rc (%)
Year 1			
Albion	Control	517.70 ± 38.55[a]	
	ALG/Zn	624.69 ± 69.08[b]	+20.67
	CS/ALG/Zn	634.85 ± 58.19[b]	+22.63
San Andreas	Control	563.24 ± 4.78[a]	
	ALG/Zn	610.79 ± 49.66[ab]	+8.44
	CS/ALG/Zn	667.13 ± 62.63[b]	+18.45
Year 2			
Albion	Control	626.10 ± 25.30[a]	
	ALG/Zn	655.63 ± 14.50[a]	+4.72
	CS/ALG/Zn	627.16 ± 18.50[a]	+0.17
San Andreas	Control	473.05 ± 53.13[a]	
	ALG/Zn	548.12 ± 50.34[ab]	+15.87
	CS/ALG/Zn	583.57 ± 23.20[b]	+23.36

*Represents a relative change (%) compared to the respective control. Values superscripted with the same letter are not significantly different according to the posthoc t-tests with Bonferroni correction ($p < 0.05$).

to pH, solubility, and oxidation-reduction state of Zn in the surrounding media also strongly influences Zn uptake by the plant (Gupta et al., 2016). Obtained results are following previously published data on TFC in strawberry juices. Šic Žlabur et al. (2019) have reported a TFC value of 659.03 mg GAE L^{-1}juice whereas here we report a range of 473.05 to 667.13 mg QE L^{-1} juice.

Interestingly, the highest increase is observed for total anthocyanins with an increase of up to 61.43% and 53.86% for both Albion and San Andreas varieties, respectively to the untreated strawberry plants (Table 3). When considering data on total anthocyanins from the literature, we can observe significantly higher values (from 503.52 to 812.83 mg M3GE L^{-1} juice). Garzon and Wrolstad (2002) reported a value of 516 mg L^{-1} of total anthocyanins in strawberry juice fortified with pelargonidin derivatives, while Tiwari et al. (2008) reported a value of 442.10 mg L^{-1} juice expressed as pelargonidin-3-*O*-glucoside. Even though significant differences are comparing to the control in the first year, in the second year of the experiment we observe a remarkable increase in the total anthocyanin content. Following a similar trend as total flavonoid content, chitosan-coated microparticles had a significantly higher influence on the synthesis of anthocyanins in strawberry fruits. When discussing flavonoids (anthocyanins) we can conclude that not only Zn^{2+} ions influence the synthesis but also the fact that CS/ALG/Zn microparticles influence the synthesis of these compounds, by lowering the pH of surrounding media.

Depending on the variety, flavan-3-ols content in strawberries differs. Oszmiański and Wojdyło (2008) compared flavan-3-ol content in different varieties and reported the values from 140.0 to 284.0 mg L^{-1}expressed as (+)-catechins. In this work, we report a range from 115.69 to 195.33 mg EE L^{-1} (Table 4). From Table 4 we can observe that only in the first year of the experiment significant differences were found between treated and untreated plants. Since the increase was not consistent throughout the 2 years of the experiment we can conclude that treatments with microparticles do not considerably influence flavan-3-ols synthesis in strawberry fruits. Flavan-3 ols are some of the metabolites of the anthocyanin biosynthetic pathway. At veraison, upstream enzymes in the flavanols biosynthetic pathway are likely to flow into the anthocyanidins biosynthetic pathway. The more transformation of flavanols into anthocyanidins may also cause the higher content of total anthocyanins (Song et al., 2015) in strawberries treated with Zn-alginate microparticles.

Antioxidant activity in strawberry fruit juices significantly increased with microparticles treatments in both varieties and experiment years (ABTS and DPPH)

Table 3. Total anthocyanins content in nontreated (control) and with microparticles (ALG/Zn and CS/ALG/Zn) treated strawberries (two varieties) – two-year soilless cultivation. Results are expressed per volume of strawberry juice.

Variety	Treatment	Total anthocyanins (mg M3GE L^{-1} juice)	*Rc (%)
Year 1			
Albion	Control	638.95 ± 47.31[a]	
	ALG/Zn	761.98 ± 4.25[b]	+19.26
	CS/ALG/Zn	744.23 ± 31.15[b]	+16.48
San Andreas	Control	523.16 ± 54.48[a]	
	ALG/Zn	625.35 ± 66.62[b]	+19.53
	CS/ALG/Zn	597.30 ± 11.62[b]	+14.17
Year 2			
Albion	Control	503.52 ± 20.98[a]	
	ALG/Zn	571.62 ± 51.15[a]	+13.52
	CS/ALG/Zn	812.83 ± 90.44[b]	+61.43
San Andreas	Control	529.50 ± 114.66[a]	
	ALG/Zn	720.43 ± 104.51[ab]	+36.06
	CS/ALG/Zn	789.67 ± 73.72[b]	+53.86

*Represents a relative change (%) compared to the respective control. Values superscripted with the same letter are not significantly different according to the posthoc t-tests with Bonferroni correction ($p < 0.05$).

Table 4. Flavan-3-ols content in nontreated and with microparticles (ALG/Zn and CS/ALG/Zn) treated strawberries (two varieties) – two-year soilless cultivation. Results are expressed per volume of strawberry juice.

Variety	Treatment	Flavan-3-ols (mg EE L^{-1})	*Rc (%)
Year 1			
Albion	Control	120.02 ± 0.71[a]	
	ALG/Zn	140.16 ± 4.55[b]	+11.75
	CS/ALG/Zn	132.48 ± 6.11[ab]	+10.38
San Andreas	Control	166.49 ± 10.01[a]	
	ALG/Zn	195.33 ± 2.25[b]	+17.32
	CS/ALG/Zn	187.97 ± 28.00[a]	+12.90
Year 2			
Albion	Control	122.61 ± 13.31[a]	
	ALG/Zn	129.66 ± 14.31[a]	+5.75
	CS/ALG/Zn	117.59 ± 6.97[a]	-4.09
San Andreas	Control	115.69 ± 14.98[a]	
	ALG/Zn	134.67 ± 3.53[a]	+16.41
	CS/ALG/Zn	125.78 ± 11.65[a]	+8.72

*Represents a relative change (%) compared to the respective control. Values superscripted with the same letter are not significantly different according to the posthoc t-tests with Bonferroni correction ($p < 0.05$).

(Tables 5 and 6). A wide range of antioxidant activity of strawberry juice has been previously reported, from 3.774 mmol TE L^{-1} juice (Šic Žlabur et al., 2019) to 27.03 mmol TEAC L^{-1} juice (Adorno et al., 2016) for ABTS and 25.84 mmol TEAC L^{-1} juice (Adorno et al., 2016) for DPPH method. Similar to the total polyphenols content, total flavonoid content, and total anthocyanins an increased antioxidant activity was found in treated strawberries. It is well known that the antioxidant activity of fruits increases with a higher share of bioactive compounds such as phenolic acids and flavonoids (Šic Žlabur et al., 2019). To reduce stress, plants produce antioxidant enzymes to keep the radical level lower than the toxic limit (Gill et al., 2015). In the short-term, soil and foliar biofortification with Zn is effective when crops have Zn deficiency-associated problems (Manzeke et al., 2014). Sida-Arreola et al. (2017) showed that the application of low-dose zinc increased concentration in the seed, regardless of the form of application. Concerning antioxidant enzymes, the application of Zn (as $ZnSO_4$ and DTPA-Zn) resulted in a significant increase in the enzyme superoxide dismutase activity.

In this research, genetically related day-neutral strawberry cultivars were used in soilless cultivation under continental climate with higher temperatures and solar insolation. Obtained results show higher values in terms of phytochemical parameters which indicates positive effects on treated plants. Previously we have reported a significant influence of treatments with microparticles loaded with chemical agents on the synthesis of secondary plant metabolites of *Vitis vinifera* L. leaves and *Lactuca sativa* L. The availability of active agents (Zn^{2+}) to the plant via encapsulation into the biopolymeric microparticles ensures its targeted and controlled release throughout plant development and maturation (Vinceković et al., 2019; Jurić et al., 2020).

4. Conclusion

Herein we report stimulation of PSM (total polyphenols, total flavonoids, total anthocyanins, and flavan-3-ols) synthesis and increase in antioxidant activity of soilless cultivated strawberries (*Fragaria* × *ananassa* Duch.) with the use of alginate microparticles (microspheres and microcapsules) loaded with a chemical agent, i.e. Zn^{2+}. Prepared microparticles (spherical, with no morphological deformities, and the size of ~1830 μm) were used for the treatment of two strawberry varieties (Albion and San Andreas). Two treatments (microspheres/microcapsules with Zn^{2+}) were carried out by applying microparticles near the root of the plant.

Both, Zn-alginate microspheres and microcapsules significantly stimulated the synthesis of PSM in

Table 5. Antioxidant activity of nontreated (control) and with microparticles (microspheres and microcapsules) treated strawberries (two varieties) determined with ABTS method – two-year soilless cultivation. Results are expressed per volume of strawberry juice.

Variety	Treatment	ABTS (mmol TE L^{-1} juice)	*Rc (%)
Year 1			
Albion	Control	7.04 ± 0.21^{a}	
	ALG/Zn	8.91 ± 0.73^{b}	+26.63
	CS/ALG/Zn	9.04 ± 0.73^{b}	+28.41
San Andreas	Control	9.68 ± 0.84^{a}	
	ALG/Zn	11.86 ± 1.06^{b}	+22.53
	CS/ALG/Zn	11.74 ± 0.33^{b}	+21.31
Year 2			
Albion	Control	8.34 ± 0.48^{ab}	
	ALG/Zn	9.12 ± 0.53^{a}	+9.36
	CS/ALG/Zn	8.18 ± 0.19^{b}	-1.96
San Andreas	Control	6.56 ± 1.51^{a}	
	ALG/Zn	7.82 ± 0.55^{ab}	+19.20
	CS/ALG/Zn	8.97 ± 0.45^{b}	+36.72

*Represents a relative change (%) compared to the respective control. Values superscripted with the same letter are not significantly different according to the posthoc t-tests with Bonferroni correction ($p < 0.05$).

Table 6. Antioxidant activity of nontreated (control) and with microparticles (ALG/Zn and CS/ALG/Zn) treated strawberries (two varieties) determined with the DPPH method – two-year soilless cultivation. Results are expressed per volume of strawberry juice.

Variety	Treatment	DPPH (mmol TE L^{-1} juice)	*Rc (%)
Year 1			
Albion	Control	5.62 ± 0.60^{a}	
	ALG/Zn	6.78 ± 0.37^{b}	+20.55
	CS/ALG/Zn	6.69 ± 0.37^{b}	+18.95
San Andreas	Control	5.91 ± 0.40^{a}	
	ALG/Zn	6.83 ± 0.09^{b}	+15.51
	CS/ALG/Zn	7.13 ± 0.61^{b}	+20.58
Year 2			
Albion	Control	6.59 ± 0.36^{a}	
	ALG/Zn	7.04 ± 0.28^{b}	+6.83
	CS/ALG/Zn	6.81 ± 0.26^{ab}	+3.34
San Andreas	Control	4.65 ± 0.97^{a}	
	ALG/Zn	6.12 ± 0.36^{b}	+31.69
	CS/ALG/Zn	6.43 ± 0.61^{b}	+38.36

*Represents a relative change (%) compared to the respective control. Values superscripted with the same letter are not significantly different according to the posthoc t-tests with Bonferroni correction ($p < 0.05$).

strawberries especially polyphenolic compounds with no visible damages on plants and fruits. Encapsulation of zinc is simple in terms of stimulating the production of PSM throughout strawberries' maturation. Microparticles keep Zn^{2+} in a readily available form. The controlled release was achieved, and the plant can uptake zinc ions passively through the root system.

Encapsulation revealed to be a sustainable, environmentally friendly, rapid, convenient, economical, and efficient for targeted delivery of chemical agents for plant nutrition and production of functional foods, strawberries fortified with PSM, that is a fruit with improved nutritional quality as potential dietary sources of natural phenolic antioxidants.

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