Scientia Horticulturae 327 (2024) 112793

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Microbial biostimulants affect the development of pathogenic microorganisms and the quality of fresh strawberries (*Fragaria ananassa* Duch.)

Magdalena Drobek [a], Justyna Cybulska [a,*], Magdalena Frąc [a], Piotr Pieczywek [a], Giorgia Pertile [a], Vadym Chibrikov [a], Artur Nosalewicz [a], Beata Feledyn-Szewczyk [b], Lidia Sas-Paszt [c], Artur Zdunek [a]

[a] *Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, Lublin 20-290, Poland*
[b] *Institute of Soil Science and Plant Cultivation State Research Institute, Czartoryskich 8, Pulawy 24-100, Poland*
[c] *Institute of Horticulture - National Research Institute, Konstytucji 3 Maja 1/3, Skierniewice 96-100, Poland*

ARTICLE INFO

Keywords:
Strawberry
Biostimulants
Maturation
Antioxidants
Microbial pathogens
Pectin
Pectinolytic enzymes

ABSTRACT

Strawberries, as non-climacteric fruits, undergo unfavourable changes after harvest such as cell damage, softening and a loss in fruit quality. One of the methods of maintaining high-quality fruit is using ecological preparations defined as biostimulants, which also eliminate the usage of pesticides and support a sustainable agriculture approach. The effectiveness of microbial preparations used as biostimulants was studied in our experiment. The use of preparations containing *Bacillus subtilis* and *Paenibacillus polymyxa* strains and bioactive substances (K4, K5 and K6) delayed the ripening processes of the Honeoye and Rumba strawberry, which resulted in a brighter colour of the skin as compared to the control (K1 I, K1 II). After applying the biopreparations, the taste was improved and the nutritional value of the strawberries increased. An average of a 14 % increase in the soluble solid content (SSC) content was observed for Honeoye strawberries treated with K4 and K6 and also the Vibrant variety treated with K6, compared to the control samples (K1 I, K1 II). The antioxidant capacity, which was determined in terms of the DPPH level in the three cultivars of the strawberries (Honeoye, Rumba and Vibrant) treated with the tested preparations, was similar and ranged between 83 and 90 %. The greatest differences in the content of antioxidants were found for anthocyanins, whose content increased by an average of 27 % in the Vibrant (K5, K6) and Rumba (K3, K5, K6) strawberries. Together, these results show that the selected preparations improve the quality of strawberries of the Honeoye, Vibrant and Rumba varieties.

1. Introduction

In recent years, there has been a growing interest in organic food production and consumption. Its key role is to provide essential nutrients and prevent food-related diseases (Ganugi et al., 2021). It should be emphasized that the production of organic food is beneficial both for consumers and the natural environment. Organic strawberries were characterized by better nutritional composition, especially larger anthocyanin content compared to conventional fruit. More attractive sensory profile because of lower acidity and higher soluble solid content were also confirmed for organic strawberries in comparison with conventionally grown fruits (Drobek et al., 2020). Differences found in the molecular architecture of cell walls between organic and conventional strawberries explain their higher resistance to mechanical damage and decay processes on harvest day (Cybulska et al., 2022).

The shift away from traditional cultivation systems requires the introduction of new organic preparations to the market. Plant biostimulants contain substances and/or microorganisms which stimulate natural processes to enhance nutrient uptake, nutrient efficiency, tolerance to abiotic stress and crop quality (Del Buono, 2021). Microbiological biostimulants are a group of preparations containing non-pathogenic and non-toxic microorganisms such as *Azotobacter* spp., *Rhizobium* spp., *Azospirillum* spp. or mycorrhizal fungi (Ganugi et al., 2021). They can be applied as seed treatment, root dip, soil amendment, or foliar spray, however, foliar spraying may be used throughout the whole season at certain growth stages to promote plant growth and

* Corresponding author.
E-mail address: j.cybulska@ipan.lublin.pl (J. Cybulska).

https://doi.org/10.1016/j.scienta.2023.112793
Received 20 October 2023; Received in revised form 15 December 2023; Accepted 18 December 2023
Available online 27 December 2023
0304-4238/© 2023 Elsevier B.V. All rights reserved.

stress resistance (Preininger et al., 2018). The applied microorganisms affect the yield and resistance of plants to both biotic and abiotic stresses and, as a consequence, the quality of the fruit (Nephali et al., 2020). In response to abiotic stresses or an attack by pathogens, plants produce defence compounds that are also health-promoting for humans (Heil and Bostock, 2002). In response to increased levels of oxidative stress caused by microbial colonization, plants secrete antioxidant compounds (phenolic compounds, terpenoids, ascorbic acid, anthocyanins, etc.) (Rouphael et al., 2020). Microbial biostimulants were applied to promote heat stress tolerance by producing Reactive Oxygen Species-degrading enzymes, reducing hydrogen peroxide content and lipidic peroxidation as well as reducing ethylene emission (Sangiorgio et al., 2020). The mechanisms of the influence of microorganisms on the plant, the production of secondary metabolites and their influence on fruit quality are complex. Beneficial organisms influence positively food quality by the accumulation of functional components which may be applied in the modulation of nutraceutical quality (Ganugi et al., 2021). It has been shown that polyphenols, which influence the colour of flowers and fruits, reduce the risk of heart disease and cancer development (Pott et al., 2019). Carotenoids, naturally occurring pigments found in most fruits and vegetables, prevent diabetes, cardiovascular disease and Alzheimer's disease (Eggersdorfer and Wyss, 2018). Ascorbic acid, on the other hand, reduces food spoilage mainly due to its antioxidant activity and low pH (Xylia et al., 2019).

The increase in the production of secondary metabolites has been proven to arise as a result of the action of microbiological biostimulants on strawberry plants. The interaction of the appropriate plant growth-promoting bacteria (PGPB) with AMF (arbuscular mycorrhizal fungi: *Funneliformis mosseae, Septoglomus visosum* and *Rhizoglomus irregulare*) improves the quality parameters of strawberries such as the anthocyanin content (Todeschini et al., 2018). The inoculation of *A. brasilense* increased the content of flavonoids and flavonols (Pii et al., 2018), while increased levels of ascorbic, p-coumaric and ellagic acids (polyphenols) were recorded in *Rhizobium* sp. treated strawberries (Flores-Félix et al., 2018). It should be emphasized that the synthesis of secondary metabolites is regulated by exposure to various stressors, signalling molecules or elicitors including physical or chemical agents, as well as microbial factors (Humbal and Pathak, 2023). For this reason, the addition of materials supporting microbial biostimulants including natural extracts, plant extracts, etc. should be promoted (Ganugi et al., 2021).

The work aims to determine the influence of newly developed biostimulants containing microorganisms and natural extracts on the quality parameters of three strawberry varieties in field conditions. Quality parameters evaluated in this experiment belonged to the factors which determine the consumer acceptance of strawberries. Non-destructive Vis-NIR spectroscopy method was used for fast determination of fruit skin appearance. Nutritional value, antioxidant properties and pectin-degrading enzymes activity were evaluated. To better understand the mechanisms of action of applied biopreparations on the most important pathogens, microorganisms on the surface of the fruit have been identified.

2. Materials and methods

2.1. Strawberry fruit

Strawberry cv. Honeoye, Vibrant, and Rumba were grown under the field experiment set up by the Institute of Soil Science and Plant Cultivation State Research Institute (Puławy, Poland) in the Agricultural Experimental Station of IUNG-PIB in Grabów (51°21′17.1″N 21°39′14.0″E). The experiment was set up on Luvisols in 4 replications in a long strip system, on experimental plots with an area of 16 m^2 (4 m x 4 m). The soil was covered with black fleece to reduce water evaporation and weed growth. The crops were irrigated 6 times during the plant growing season (27/04/2020, 29/04/2020, 15/05/2020, 19/05/2020, 22/05/2020, 15/06/2020) with 6000–7000 l of water during each irrigation for the entire experimental plantation with an area of approximately 12 ares (1200 m^2). Strawberries were harvested manually on 08/06/2020. Soil, plant, and fruit samples were collected during the second season on strawberry growth. The strawberries were treated with five organic preparations containing, among others, microorganisms originating from the SYMBIOBANK collection of the Research Institute of Horticulture in Skierniewice and plant extracts (Table 1). The microbes were applied as a component of solid or liquid formulations of biopreparations. The solid preparation (P3) and the carrier were applied in the form of pellets under the plant roots once (20/05/2020) during the growing season. The P3 preparation was applied to treatments K2, K3, K4, and K5, while the carrier to K1II treatment. Liquid biopreparations (P1 and P2) were used twice (29/05/2020; 29/06/2020) during the plant growing season by spraying the plants. On the same dates, plants in control objects were sprayed with water (K1I) and with the carrier of liquid biopreparations (K1II). Biopreparation P1 was applied in treatments K3, K5, and K6, while biopreparation P2 was used in treatments K4, K5 and K6.

2.2. Vis-NIR spectroscopy and data analysis

Changes in fruit reflectance in response to applied biopreparations were carried out with a CI-710 Miniature Leaf Spectrometer (CID Bio-Science, 1554 NE 3rd Avenue, Camas, WA USA, 98,607). The device was operated with SpectraSuite® Spectrometer Operating Software (Ocean Insights, https://www.oceaninsight.com/). The device was calibrated by the manufacturer's instructions, using the manufacturer's calibration standard. Reflectance spectra were measured within the 450–1000 nm spectral range. The single spectrum acquisition time was set to 600 ms. Three averaging scans were made during each measurement, with an additional boxcar averaging filter turned on (with a half-width of three data points). Measurements were performed in 10

Table 1
Composition of applied preparations.

Component	K1I	K1II	K2	K3	K4	K5	K6
Carrier (extract from nettle, horsetail, and calendula, liquid humic acids, Vinasa-yeast culture effluent, bran, dry humic acids, mustard, rapeseed oil)	Control – no treatment	x					
Bacillus subtilis (B4/19) AF75AB2 and *Bacillus* sp. (B7/19) Sp115AD, extracts (nettle, horsetail, calendula), liquid humic acids, foliar application: 10^5 CFU/cm^2; soil application: 10^8 CFU/plant, liquid formulation (P1)				x		x	x
Paenibacillus polymyxa (B13/19) Sp116AC*, *Bacillus* sp. (B7/19) Sp115AD, liquid humic acids, Vinasa (yeast culture effluent), foliar application: 10^5 CFU/cm^2, soil application: 10^8 CFU/plant, liquid formulation (P2)					x	x	x
Bacillus subtilis (B4/19) AF75AB2, *Bacillus* sp. (B6/19) AF75BC, bran, dry humic acids, mustard, rapeseed oil, 10^9 CFU/plant, solid preparation (P3)			x	x	x	x	

The "x" sign indicates the presence of the preparation component.

replicates for each treatment and cultivar (210 fruit in total), near the equatorial area on the surface of the fruit. The visual range of the spectral data was used to calculate the colour characteristics of the sampled strawberries, expressed through the CIE L*a*b colour space. Spectral data analysis was carried out using the ChemoSpec R package (Hanson, 2016). Before analysis, the reflectance spectra were cropped to the 500–1000 nm wavelength range due to the high level of noise close to the limits of the measuring range. To avoid the unwanted effects of spectroscopic measurements (baseline shift due to lamp or detector instabilities, the effects of fruit curvature and repositioning) a derivative spectroscopy approach was applied to the reflectance spectra. The first derivative spectra were calculated using a Savitzky-Golay filter (filter order = 2, length = 41) to eliminate baseline shifts and show the rate of change of reflectance with respect to wavelength. Then a principal component analysis was performed using the first derivative spectra (derivative spectra were normalized using the Standard Normal Variate method).

2.3. Strawberry fruit postharvest quality indicators

2.3.1. Dry weight

0.5 g of strawberries was dried at 105 °C until a constant weight was achieved. The dry matter content in the individual treatments was expressed as a percentage of fresh weight (FW). The result is presented in terms of the mean of three replicates.

2.3.2. Acidity

The acidity of the fruit was determined according to the Nunes et al. (1995) procedure. 6 g of strawberry juice was diluted by adding 10 ml of deionized water. The solution was titrated with 0.1 M NaOH until the pH approached 8.1. The acidity of the solution was expressed in terms of the percentage of citric acid according to the following formula: [(volume of NaOH • 0.1 • 0.064/6 g of juice) • 100]. The result is given as the mean of three replicates.

2.3.3. Soluble solid content

The soluble solid content (SSC) was determined using a refractometer (PAL-BX/RI, ATAGO, Tokyo, Japan) by directly dropping fresh juice which was placed on the prism of the refractometer. The SSC was expressed in percentage (%). The result is given as the mean of three replicates.

2.3.4. Total anthocyanin content

The content of anthocyanins in strawberries was determined according to the method developed by Spayd and Morris (1981) and da Silva et al. (2007). 18 ml of 0.5 % (v/v) HCl in methanol was added to 2 g of the strawberry pulp. The mixture was incubated at 4 °C for 1 h in the dark to extract the pigment. The next step was the centrifugation of the mixture and the spectrophotometric measurement of the anthocyanin content at a wavelength of 520 nm. The anthocyanin content was calculated from the following equation: (A520 • dilution factor • (molecular weight (MW) of PGN/molar extinction coefficient); MW_{PGN} = 433.2 and the molar extinction coefficient = 2.908 • 104). The anthocyanin content was expressed in terms of the pelargonidin-3-glucoside (PGN) content in mg 100 g^{-1} FW. The result is given as the mean of three replicates.

2.3.5. Total soluble polyphenol content

The content of polyphenolic compounds was determined according to Sim et al. (2010). 1.58 ml of water and 0.1 ml of Folin-Ciocalteu reagent were added to 0.02 ml of strawberry juice.

The solution was mixed for 3 min and then 0.3 ml of saturated sodium carbonate was added. Then the mixture was incubated for 30 min at 40 °C in the dark to obtain a persistent characteristic blue colour. After the solution had cooled down, the absorbance was measured at a wavelength of 765 nm. The strawberry polyphenol content was calculated against gallic acid standards and expressed in terms of mg 100 g^{-1} FW. The result is given as the mean of three replicates.

2.3.6. The content of vitamin C

The content of vitamin C was determined using the titration method (PN-A-04019, 1998). Fifty grams of fruit was blended in the presence of an extraction solution (2 % oxalic acid) and filtered. Ten millilitres of the filtrate was immediately titrated with a solution of 2,6-dichlorophenol until a pale pink colouration was obtained. The content of vitamin C was expressed in mg 100 g^{-1} FW. The result is given as the mean of three replicates.

2.3.7. The content of malondialdehyde

The procedure for determining the content of malondialdehyde (MDA) in strawberries was carried out according to Liu et al. (2018) with some changes. 0.1 g of strawberries was homogenized in the presence of 0.1 % trichloroacetic acid and incubated in an ice bath for 10 min. The mixture was centrifuged (1800 x g, 10 min, 4 °C) and the supernatant was collected. 1 ml of a solution of 0.67 % thiobarbituric acid in 10 % trichloroacetic acid was added to 0.05 ml of the supernatant. The mixture was incubated at 95 °C for 15 min, and then rapidly cooled in an ice bath and centrifuged (1800 × g, 10 min, 4 °C). The absorbance of the supernatant was measured at 430, 532 and 600 nm. The MDA content was calculated using the following formulas: C [μmol L^{-1}]= 6.45 • (A_{532} - A_{600}) - 0.56 • A_{430}, MDA content [μmol kg^{-1}]= (C • V)/(Vs • W • 1000)]; C- MDA concentration in the reaction mixture, V- total sample volume [ml], Vs- volume of the sample extract solution taken for the reaction [ml], W- sample weight [kg]. The MDA content was expressed in terms of μmol kg^{-1} FW. The result is given as the mean of three replicates.

2.3.8. Antioxidant capacity

The antioxidant capacity of the strawberries was determined by measuring the content of (2,2-diphenyl-1-picrylhydrazyl (DPPH) using the method proposed by Hangun-Balkir and McKenney (2012). 5 g of strawberries were homogenised in the presence of 20 ml of 80 % ethanol for 20 min. The mixture was centrifuged (1800 x g, 15 min, 4 °C). 2 ml of DPPH reagent (0.010 g DPPH was dissolved in 100 ml of 80 % ethanol) was added to 2 ml of supernatant. The solution was incubated at room temperature for 30 min. The absorbance was measured at 517 nm. Antioxidant capacity was expressed as a percentage of DPPH scavenging capacity according to the following formula: DPPH [%] = ((A control-A sample))/(A control) • 100. The result is given as the mean of three replicates.

2.3.9. Enzyme activity

The enzymatic activity of the strawberries was determined according to Wei et al. (2010). The enzyme extract was prepared from 3 g of strawberry pulp. 6 ml of 12 % polyethylene glycol containing 0.2 % sodium bisulphate was added to the pulp and the resulting mixture was centrifuged (21,000 x g, 10 min). The supernatant was discarded and 6 ml of 0.2 % sodium bisulphate was added to the residue. Then the mixture was centrifuged (21,000 x g, 10 min) again and the supernatant was discarded. 6 ml of an extraction solution containing 0.1 M sodium acetate, 100 mM sodium chloride, 2 % mercaptoethanol and 5 % polyvinylpyrrolidone in a ratio of (1: 1: 1: 1) was added to the residue. The mixture was incubated at 4 °C for 1 h to extract the enzymes and then centrifuged (21,000 x g, 10 min). The supernatant was an enzyme extract used in the analyses below.

To determine the enzymatic activity of polygalacturonase (PG) 0.8 ml of 0.5 % polygalacturonic acid (in 50 mM sodium acetate buffer, pH= 5.2) was added to 0.2 ml of the enzyme extract. The mixture was incubated at 37 °C for 2 h. 2 ml of 0.1 M borate buffer (pH= 9) and 0.3 ml of 1 M cyanoacetamide were added to the reaction mixture. The mixture was placed in a water bath (100 °C) for 10 min. Absorbance was measured at 276 nm. Galacturonic acid was used as a standard. The

enzymatic activity of PG was determined in μg g^{-1} min^{-1}. The result is given as the mean of three replicates.

To determine the enzymatic activity of pectin methylesterase (PME) 1 ml of the enzyme extract was added to 4 ml of 1 % (w/v) citrus pectin. The pH of the solution was measured and incubated at 37 °C for 1 h. The sample was titrated with 0.01 M sodium hydroxide to pH= 7.4. The enzymatic activity of PME was expressed in μmol g^{-1} min^{-1}. The result is given as the mean of three replicates.

To determine the enzymatic activity of α-L-arabinofuranosidase (AF) and β-galactosidase (B-Gal) 0.5 ml of the appropriate substrate (3 mM p-nitrophenyl-α-D-arabinofuranosidase or 3 mM p-nitrophenyl-β-D-galactopyranosidase) was added to 0.5 ml of 0.1 M sodium acetate. The mixture was incubated at 40 °C for 10 min, then 0.5 ml of enzyme extract was added and the mixture was incubated at 37 °C for 30 min. 2 ml of 0.5 M sodium carbonate was added to the mixture and the absorbance was immediately measured at 276 nm. p-nitrophenyl was used as a reference. The enzymatic activity of AF and B-Gal was expressed in terms of μmol g^{-1} min^{-1}. The result is given as the mean of three replicates.

2.4. Microbiological assessment

2.4.1. Fungal and fungal-like pathogen detection in environmental samples

The presence of the key pathogens of strawberry plantations (*Botrytis cinerea*, *Colletotrichum* sp., *Phytophthora* sp. and *Verticillium* sp.) was evaluated using PCR methods. DNA extraction from strawberry fruit, leaves and soil was performed through the use of a Soil DNA Purification kit (EURx, Poland). For each pathogen specific primers were used for the amplification of functional genes as set out in the relevant patent application (**P.431989; P.431991; P.431992; P.431993**).

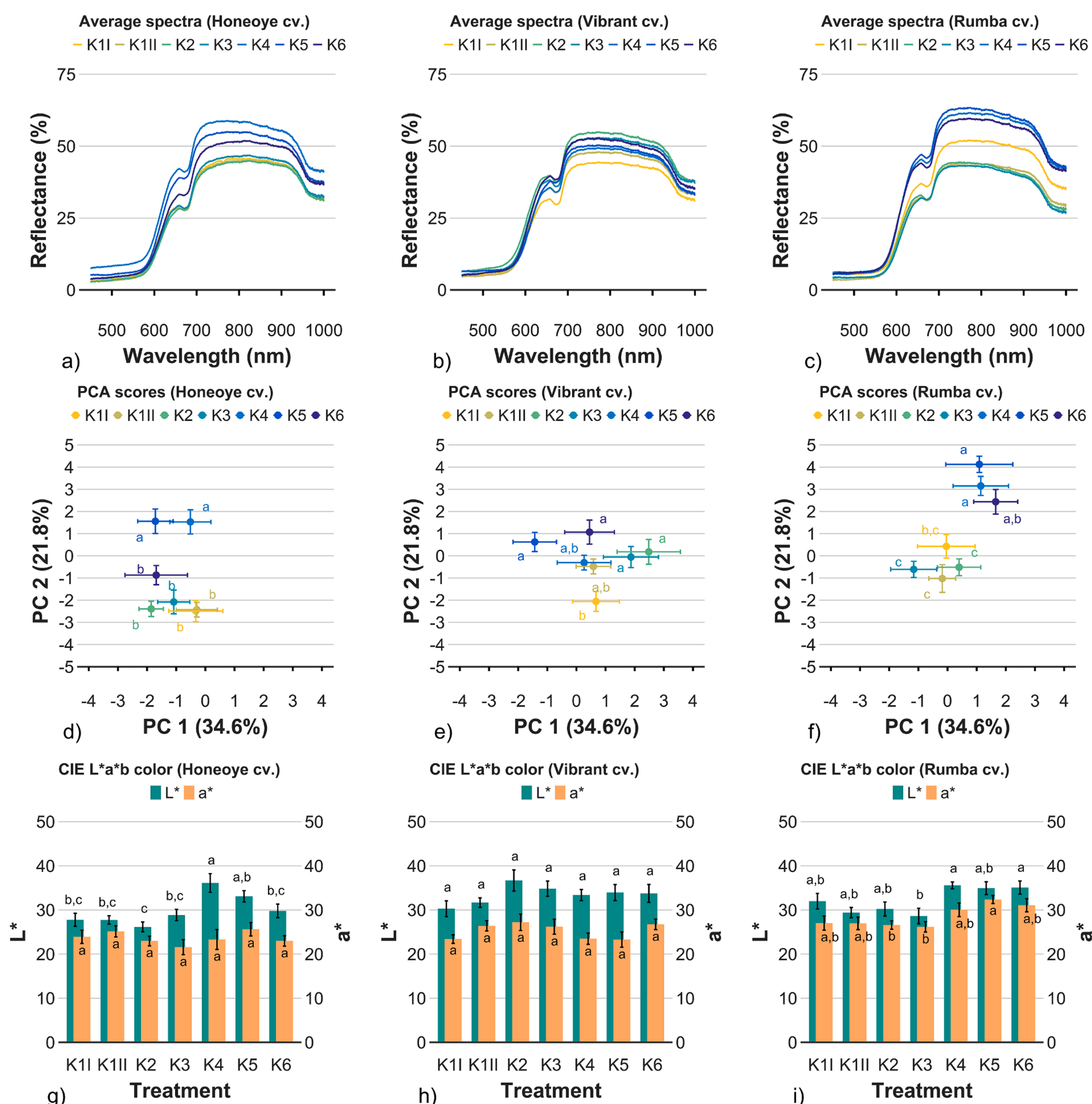

Fig. 1. Averaged reflectance spectra of strawberries (cv. Honeoye, Vibrant and Rumba) subjected to different treatments (a–c). The spectra were averaged over ten fruits for each treatment separately. The middle row presents the principal component score plots for three strawberry fruit cultivars (d–f) obtained from a principal component analysis of the reflectance spectra. Two-dimensional score plots present average values of PC 1 and PC 2 for each treatment (points) with standard errors (bars). The values in parentheses represent the percentage of explained variance. The bottom rows (g–i) show the strawberry skin colour characteristics expressed in the CIE L*a*b colour space. Data points with a different letter are significantly different (One-way ANOVA, Tukey HSD, $p < 0.05$).

2.4.2. Bacterial and fungal total count in environmental samples

For each type of sample (soil, leaves, fruit) 1 g of each replication was weighed and mixed with 9 ml of sterile water and shaken in a rotator for 1 h. Then 10-fold dilutions were prepared and the appropriate dilutions were selected to evaluate the total count of bacteria and fungi using a Plate Count Agar medium (PCA) and Bengal Rose medium (BR), respectively. 100 μl of inoculum was spread over the surface of the medium in Petri dishes. All of the Petri dishes were incubated at 26 °C and the colonies were counted at 2 to 5 incubation days. The experiment was performed in 3 replications.

2.5. Statistical analysis

The data obtained were analysed using STATISTICA software (Statistica v.12, StatSoft Inc., Tulsa, OK, USA). Data were analysed using a two-way analysis of variance (ANOVA) followed by a post hoc HSD Tukey test, significant differences were determined at $p < 0.05$. The samples for the tests were prepared in no less than triplicate quantities.

3. Results

3.1. Vis-NIR spectroscopy and data analysis

The averaged reflectance spectra of strawberries (cv. Honeoye, Vibrant and Rumba) for applied preparations, collected over a wavelength range of 450–1000 nm are shown in Fig. 1a–c. In general, the reflectance curves of individual strawberries were smooth over the entire spectral region. In all cases, a reflectance peak was observed within the 630–690 nm spectral range. The differences between the averaged spectra were visible in the reflectance values covering a broad wavelength range from 450 to 900 nm. The highest differences were observed for the Honeoye and Rumba cultivars, whereas preparations K4, K5 and K6, showed higher reflectance values than the other treatments.

The reduction of undesired effects of baseline shifts related to the curved surface of the fruit and repositioning, as well as the improvement in data discriminatory power, were achieved using the derivative spectroscopy approach, applied to reflectance spectra. Subsequently, data was subjected to PCA analysis the results of which are presented as principal component score plots for three strawberry fruit cultivars in Fig. 1d–f. Even though the first principal component (PC 1) explained the largest part of the total variability of the sample (34.6 %), its discriminatory capabilities were low. This was indicated by the ANOVA results, which showed no significant differences between the preparations (data not shown). The second component (PC 2) was correlated with the strawberry spectral features responsible for the differences between the tested samples, showing a high discriminatory power (letter indexes in Fig. 1d–f). The PCA loadings indicated the high degree of sensitivity of PC 2 to reflectance variations (slope, peak width etc.) within the range of 580 – 690 nm, which corresponds mainly to changes in chlorophyll content (Xiaobo et al., 2011). A negative and weaker correlation was also found for PC 2 scores and reflectance variations in the 930 – 960 nm spectral band, which relates to the water content in the fruit (Ma et al., 2016). Since the analysis was based on the first derivatives of the spectra, which were quite smooth, changes in the spectral range from 690 to 910 nm showed an absence of or only a slight negative correlation with PC 2. The ANOVA results for PC 2 confirmed the initial conclusions based on examinations of the raw spectra. Despite a partial group overlap and variation among the cultivars, preparations K1I, K1II, K2 and K3 showed different spectral characteristics than preparations K4, K5 and partially K6. A PCA analysis indicated that the score plots assigned to K1I, K1II, K2 and K3 were shifted towards the negative values on the PC2 axis and this group were separated from the group of K4, K5 and K6 preparations that positioned mostly on the positive values of the PC2. This effect was visible for the Honeoye and Rumba cultivars for the K4 and K5 preparations (Fig. 1d and 1f). In the case of the Rumba cv. PC 2 values for the K6 treatment were significantly higher than for the K1II, K2, and K3 preparations (Fig. 1f), however, they showed no difference compared to the K1I control sample. The smallest variation in PC 2 values was observed for the Vibrant variety. In this case, the strawberry spectral data after the application of K2, K3, K5 and K6 preparations was significantly different from the K1I control sample, but not K1II (containing carrier). The characteristics of strawberry skin colour varied with respect to the luminosity component (Fig. 1g–i). Increased luminosity (brightness) was reported for K4, and K5 and also for the K4, K5 and K6 treated strawberries of the Honeoye and Rumba cultivars, respectively. For the Vibrant cultivar increased L* values were reported for the K2, K3, K4, K5 and K6 preparations, however, the changes were not statistically significant. At the same time the colour component expressed by the values along the a* axis (relative to the green–red opponent colours) showed no significant changes, except for the Rumba cultivar where a moderate shift towards a more intensive red skin colour was reported for the K5 and K6 treatments.

3.2. Strawberry fruit postharvest quality indicators

Figs. 2 and 3 show the effect of the treatment (for all cultivars) on strawberry fruit postharvest quality indicators and enzyme activity levels, respectively. Table 2 shows the combined effect of the treatment and the cultivar used on the resulting values of strawberry fruit postharvest quality indicators.

The overall response of the strawberry fruit in terms of SSC levels was a decrease in this parameter after the application of K1II, K2 and K3 treatments, compared to the K1I control (Fig. 2a). The SSC of the strawberries treated with K4, K5 and K6 preparations remained at the same level as the control sample K1I. The opposite trends were reported for acidity. The acidity of the K2, K3 and K4 treated samples increased, while K1II, K5 and K6 showed lower values when compared to the K1I control. Although slightly smaller, the differences in the content of anthocyanins between the K2-K6 treatments were statistically insignificant compared to the control samples (K1I and K1II). Significantly higher amounts of polyphenols compared with the remaining treatments were reported for the K5 and K6-treated strawberries (Fig. 2b). Variations in DPPH (Fig. 2c) and Vitamin C (Fig. 2d) levels were insignificant among the applied treatments. The MDA levels of the K4 and K5 treated strawberries showed high and significant increases concerning the K1I and K1II control samples. A smaller but also significant increase in MDA was also reported for the K2 and K6 preparations. The average dry weight (DW) of the strawberries for all of the tested cultivars and preparations varied within the range of 3.9–10.5 % of FW. The applied treatments did not change the dry weight of the strawberries (Fig. 2d). In considering the data for all of the cultivars together, the activity of the pectinolytic enzymes showed no statistical differences with respect to the applied preparations (Fig. 3). Only in the case of polygalacturonase were higher (but not significantly higher) activity levels observed after the application of K1II, K2, K3 and K4 preparations.

Despite showing a greater degree of variability within separate groups, the trends in the effects of the applied treatments studied for each cultivar individually (cultivar x treatment in Table 2) were in line with the general findings reported for all of the cultivars together. Despite the occurrence of a relatively high total variance, the analysis of within-group variance indicated no significant differences between the DW of strawberries subjected to different preparations. Similarly, no significant changes in the content of polyphenols were observed for each of the tested strawberry cultivars. The applied preparations only induced marginal changes in the antioxidant capacity of the strawberries. Nonetheless, the K3 and K5 preparations resulted in an increase in DPPH for Honeoye, while K5 decreased the antioxidant capacity for Vibrant strawberries. The changes in vitamin C content were limited to the Vibrant cultivar only, similar to the DPPH level being a result of the bio-variability of the sample rather than a trend induced by general treatment. For K2 and K4 the vitamin C content increased, while for K3

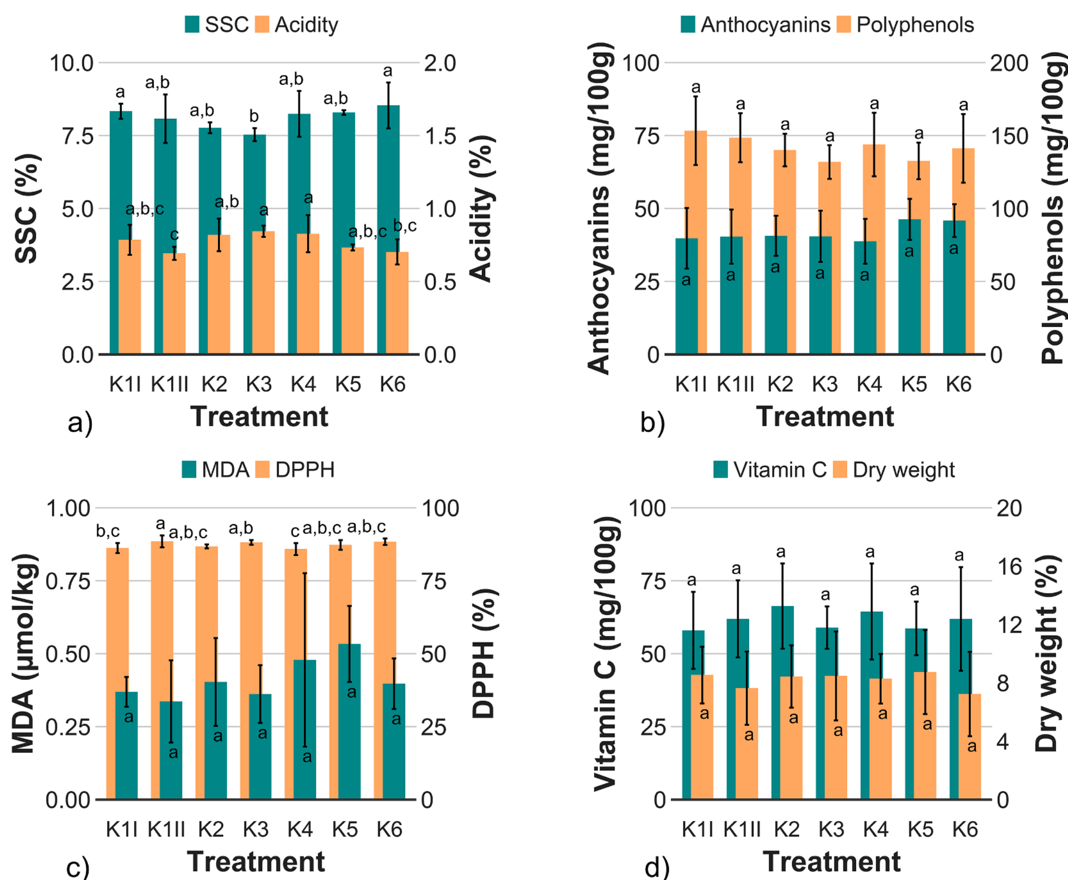

Fig. 2. Effect of treatment on strawberry fruit postharvest quality indicators (for all cultivars): (a) SSC and acidity, (b) anthocyanins and polyphenols, (c) MDA and DPPH, (d) vitamin C and dry weight. Data points show average values with 95 % confidence intervals (bars). Data points with different letter indices are significantly different (One-way ANOVA, Tukey HSD, $p < 0.05$).

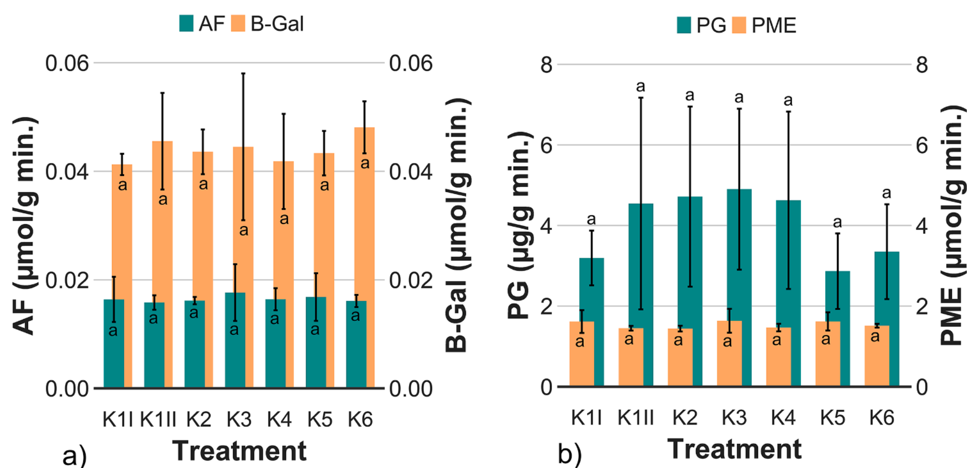

Fig. 3. Effect of the treatment of strawberry fruit on the enzyme activity levels (for all cultivars): (a) α-L-arabinofuranosidase (AF) and β-galactosidase (B-Gal), (b) polygalacturonase (PG) and pectin methylesterase (PME). Data points show the average values with 95 % confidence intervals (bars). Data points with different letter indices are significantly different (One-way ANOVA, Tukey HSD, $p < 0.05$).

and K6 it decreased when compared to the control samples (K1I and K1II).

Significant differences between preparations were reported for indicators relevant to consumers and affecting the taste of fruit - acidity and SSC (Table 2). Compared to the control sample (K1I) an increase in the acidity of strawberries was reported for K2 and K4 preparations for the Honeoye cultivar. Similar results were obtained for K2, K3, K4 and K5 preparations for Vibrant cv., and also for the K3 preparation for the Rumba cv. Significantly lower or similar values were observed for the remaining treatments and the corresponding varieties. For the Honeoye cultivar, the SSC content for the K4, K5 and K6 preparations was higher than in the case of the control samples and the remaining preparations. In the case of Vibrant cv. an increase in SSC with respect to the K1I treatment was observed only in the case of the K6 preparation, with

Table 2
The combined effect of the cultivar and applied treatment on strawberry fruit with reference to postharvest quality indicators (table shows mean values with standard deviations in brackets).

Cultivar	Treatment	Dry matter content (%)	SSC (%)	Acidity (%)	Polyphenols (mg/100 g FW)	Anthocyanins (mg/100 g FW)	Vitamin C (mg/100 g FW)	DPPH (%)	MDA (μmol/kg FW)
Honeoye	K1I	7.40 ^{B,C,D} (0.77)	8.00 ^{A,B} (0.00)	0.92 ^{A} (0.01)	130.69 ^{A,B} (3.59)	53.18 ^{A} (2.46)	64.62 ^{CE,E} (0.32)	84.01 ^{A} (0.43)	1.56 ^{G,H,I} (0.10)
	K1II	8.10 $^{C, D, E}$ (0.39)	7.83 $^{A, C}$ (0.06)	0.74 ^{B} (0.01)	146.38 $^{B, C, D, E}$ (9.40)	52.51 ^{A} (2.05)	72.53 $^{F, G}$ (2.50)	85.89 ^{B} (0.25)	1.49$^{F, G, H}$ (0.09)
	K2	5.33 ^{A} (0.56)	8.00 $^{A, B}$ (0.10)	0.96 ^{C} (0.00)	132.61 $^{A, B, C}$ (2.90)	48.71 ^{A} (0.36)	73.78 $^{F, G, H}$ (0.56)	86.65 $^{B, C}$ (0.37)	0.96^{C} (0.07)
	K3	7.82 $^{C, D, E}$ (0.08)	7.57 ^{C} (0.06)	0.89 ^{D} (0.00)	121.83 $^{A, B}$ (6.89)	51.70 ^{A} (2.84)	64.53 $^{D, E}$ (0.59)	87.61 $^{C, D}$ (0.25)	1.01 $^{C, D}$ (0.07)
	K4	8.49 $^{C, D, E}$ (0.45)	9.07 ^{E} (0.25)	0.99 ^{E} (0.02)	134.30 $^{A, B, C}$ (3.51)	47.13 ^{A} (0.81)	60.00 $^{C, D}$ (0.68)	83.20 ^{A} (0.85)	0.34^{A} (0.03)
	K5	5.31 ^{A} (0.36)	8.30 $^{B, D}$ (0.00)	0.75 ^{B} (0.00)	140.22 $^{A, B, C, D}$ (8.74)	48.31 ^{A} (4.34)	58.65 ^{C} (1.49)	88.91 ^{D} (0.09)	1.43 $^{F, G}$ (0.02)
	K6	5.59 $^{A, B}$ (0.33)	8.40 ^{D} (0.00)	0.80 ^{F} (0.01)	142.24 $^{B, C, D}$ (6.23)	51.88 ^{A} (2.01)	75.65 $^{G, H}$ (3.75)	88.37 $^{B, C}$ (2.10)	1.06 $^{C, D, E}$ (0.04)
Vibrant	K1I	9.74 ^{E} (0.45)	8.43 ^{A} (0.06)	0.71 ^{A} (0.00)	163.02 $^{D, E}$ (9.02)	34.70 $^{A, B}$ (3.67)	68.77 $^{E, F}$ (0.99)	87.45 $^{A, B}$ (0.80)	1.69 $^{I, J, K}$ (0.03)
	K1II	8.15 $^{C, D}$ (0.61)	7.27 ^{B} (0.15)	0.70 ^{A} (0.01)	163.28 $^{D, E}$ (14.84)	35.59 $^{A, B, C}$ (0.73)	68.67 $^{E, F}$ (0.99)	89.12 ^{C} (0.09)	0.77 ^{B} (0.05)
	K2	9.27 $^{D, E}$ (0.39)	7.63 ^{C} (0.06)	0.80 ^{B} (0.00)	143.04 $^{B, C, D}$ (4.94)	40.35 ^{C} (0.23)	78.12 ^{H} (0.72)	87.45 $^{A, B}$ (0.09)	1.59 $^{H, I}$ (0.03)
	K3	8.27 $^{C, D, E}$ (0.86)	7.27 ^{B} (0.06)	0.83 ^{D} (0.00)	139.64 $^{A, B, C, D}$ (13.32)	32.49 ^{A} (0.67)	62.99 $^{C, D}$ (0.28)	89.12 ^{C} (0.09)	1.36 ^{F} (0.00)
	K4	8.60 $^{C, D,E}$ (0.88)	8.37 ^{A} (0.06)	0.79 ^{B} (0.00)	170.77 ^{E} (12.44)	39.53 $^{B, C}$ (1.49)	85.17 ^{I} (0.64)	87.18 ^{A} (0.25)	3.21 ^{M} (0.03)
	K5	7.61 ^{C} (0.48)	8.23 ^{A} (0.12)	0.74 ^{C} (0.00)	144.04 $^{B, C, D}$ (5.60)	53.10 ^{D} (0.45)	69.02 $^{E, F}$ (0.27)	85.25 ^{D} (0.67)	2.01 ^{L} (0.02)
	K6	8.64 $^{C, D, E}$ (0.46)	9.50 ^{D} (0.00)	0.70 ^{A} (0.00)	163.42 $^{D, E}$ (7.54)	46.16 ^{E} (2.09)	75.66 $^{G, H}$ (4.35)	88.69 $^{B, C}$ (0.32)	1.13^{F} (0.05)
Rumba	K1I	8.71 ^{C,D,E} (0.79)	8.57 ^{A} (0.06)	0.73 ^{A} (0.00)	156.74 ^{C,D,E} (14.72)	31.49 ^{A} (3.67)	40.63 ^{A} (0.99)	87.18 ^{A,B} (0.80)	1.43 ^{F,G} (0.00)
	K1II	8.91 ^{C,D,E} (0.22)	9.13 ^{B} (0.15)	0.64 ^{B} (0.01)	129.49 ^{A,B} (4.11)	32.97 ^{A} (0.73)	44.58 ^{A,B} (0.99)	90.45 ^{C} (0.09)	1.19 ^{E} (0.00)
	K2	9.00 ^{C,D,E} (0.20)	7.67 ^{C} (0.06)	0.70 ^{C} (0.00)	134.48 ^{A,B,C} (3.20)	32.94 ^{A} (0.23)	47.02 ^{B} (0.72)	86.17 ^{B} (0.09)	1.66 ^{I,J} (0.02)
	K3	9.05 $^{C, D, E}$ (0.47)	7.77 ^{C} (0.06)	0.81 ^{D} (0.00)	134.26 $^{A, B, C}$ (7.71)	37.23 ^{B} (0.67)	49.33 ^{B} (0.28)	87.72 ^{A} (0.09)	1.46 $^{F, G, H}$ (0.02)
	K4	8.52 ^{C,D,E} (0.17)	7.30 ^{D} (0.06)	0.70 ^{C} (0.00)	126.50 ^{A,B} (7.52)	29.61 ^{A} (1.49)	48.13 ^{B} (0.64)	87.15 ^{A,B} (0.25)	1.79^{J,K} (0.00)
	K5	8.48 ^{C,D,E} (0.30)	8.33 ^{E} (0.12)	0.71 ^{C} (0.00)	122.91 ^{A,B} (2.22)	38.04 ^{B} (0.45)	47.88 ^{B} (0.27)	87.67 ^{A} (0.67)	1.81 ^{K} (0.00)
	K6	8.97 $^{C, D, E}$ (0.47)	7.70 ^{C} (0.00)	0.61 ^{E} (0.00)	115.92 ^{A} (4.50)	39.48 ^{B} (2.09)	45.57 $^{A, B}$ (23.55)	88.08 ^{A} (0.32)	2.05 ^{L} (0.03)

Data points with different letter indices are significantly different (Two-way ANOVA, Tukey HSD, $p < 0.05$). Significant differences between control samples (K1I and K1II) and applied treatments were highlighted in red for each cultivar individually.

preparations K1II, K2, and K3 showing lower values of SSC and K4, K5 remaining at the same level of SSC as K1I. For the Rumba cv. all preparations showed significantly lower SSC values as compared to the K1I and K1II treatments.

A moderate effect of the applied treatments was reported about the content of anthocyanins. An increase in the concentration of anthocyanins was reported in the case of the K5 and K6 preparations for the Vibrant and Rumba strawberries and for the K3 preparation for Rumba strawberries only when compared to both of the control samples. For the Honeoye cultivar, the content of anthocyanins did not change between treatments. A large degree of variation in the levels of oxidative stress marker – MDA – concerning the preparation applied was reported

Table 3
An average number of bacterial and fungal microorganisms expressed in terms of x10^3 cfu (colony-forming units) for strawberry fruit.

		Treatment						
		K1I	K1II	K2	K3	K4	K5	K6
Bacteria	Honeoye	4.3	19.3	11.0	5.3	NA	5.0	16.0
	Vibrant	13.3	9.0	11.3	8.3	6.0	8.3	12.7
	Rumba	26.5	52.5	16.3	8.7	5.7	11.5	31.7
Fungi	Honeoye	6.0	10.7	5.0	3.7	NA	7.0	12.0
	Vibrant	11.3	6.7	11.0	11.0	6.0	15.3	15.7
	Rumba	19.7	50.3	21.3	5.7	5.7	7.0	23.3

Data points with different letter indices are significantly different (Two-way ANOVA, Tukey HSD, $p < 0.05$). The colour scale applied to individual cells corresponds to the range of the observed values (with red corresponding to high values and green corresponding to low values, NA-not appear).

in the case of all of the strawberry cultivars. The K2, K3 and K4 preparations significantly decreased the level of MDA in the Honeoye strawberries as compared to the K1I and K1II control samples (Table 2), with K1I showing the highest levels of MDA among all of the treatments tested. The opposite response was reported for the Vibrant and Rumba strawberries, where MDA increased for the K4 and K5 preparations, and also for the K2, K4, K5 and K6 preparations for the Vibrant and Rumba cultivars respectively.

Tables 3 and 4 show the results of the microbiological assessment of three strawberry cultivars.

The microbiological assessment of the strawberries indicated a lower number of fungal and bacterial microorganisms in the case of fruit treated with K3 and K4 and in part with K5 (Table 3) preparations. This effect was similar for both types of microorganisms but varied depending on the strawberry cultivar used, being more pronounced for Honeoye and Rumba cv. The number of fungi and bacteria measured in the control (K1I) was higher than that for K1II for the Vibrant cultivar, while the carrier used in the K1II samples caused a drastic increase in the number of microorganisms for the Honeoye and Rumba cv.

In considering the individual varieties, the lowest number of microorganisms was reported for Honeoye strawberries, while the Rumba cv. showed the highest contamination. This relationship was preserved even when omitting the high outliers reported for the Rumba cv. that was treated with the K1II preparation.

For all of the strawberry varieties and treatments applied, PCR detection produced negative results for all four major pathogens for samples taken from the fruits. Regardless of the sampling site, the lowest detection rate was recorded for the *Colletotrichum* sp. pathogen. The highest detection rate of all pathogens was reported for soil samples collected from the root zone of the plant. For all strawberry cultivars, the average detection rate of *Phytophthora* sp. and *Verticillium* sp. pathogens in the soil samples was higher for the control samples (average from K1I and K1II) than the detection rate averaged for all of the remaining treatments. For the *Botrytis cinerea* pathogen, a similar effect was only observed in the case of Rumba strawberries. For Vibrant cv. the average detection rate of *Botrytis cinerea* for the control samples was lower when compared to the remaining treatments. For Honeoye strawberries, in both cases the detection rate was similar.

4. Discussion

The collected spectra bands reflected information concerning the physical state and chemical content of the strawberries. Since the fruits were grown under the same environmental conditions (field location, irrigation and fertilization), the differences in the averaged spectra for individual cultivars indicate chemical and biological responses to the applied treatments. It was shown for overripe strawberry fruit that an absorption band near 660 nm disappears with the degradation of chlorophyll (Gao et al., 2020). Moreover, the ripening of the strawberry fruit was associated with a high content of anthocyanins, which show an absorption maximum near 520 nm (Liu et al., 2014). In this study, fruit spectra were characterized by a low reflectance (high absorption) near the 520 nm band responsible for anthocyanins, and the clear presence of a chlorophyll absorption band near 660 nm. Therefore it was concluded that strawberry fruit were collected for the most part at the early ripe and ripe maturity stage (Gao et al., 2020).

Although they were collected at the same time, the strawberries showed clear differences in their spectral response over the entire studied spectral range. Multiple studies have shown a general downward trend in reflectance values between 500 and 950 nm with the progress in the degree of strawberry maturity. As mentioned above, the 520 nm and 660 nm absorption bands depict changes in anthocyanins and chlorophyll content, reflectance within 750–1000 nm reflects the simultaneous changes in soluble solids content, cell wall structure (cellulose, pectin matrix) and water content (Yan et al., 2017). In the study on "Hongyan" strawberries Weng et al. (2020) observed a positive correlation between reflectance and SSC content in the 750–950 nm range. Our study, however, shows such trends only in cv. Honeoye and Vibrant suggest a cultivar-specific relationship between SSC and reflectance. Strawberry fruits are characterised by low within-season stability of SSC and high genotype variability as indicated in studies on 410 strawberry genotypes by Hasing et al. (2013). In addition, spectral data analysis showed that the differentiation of the samples occurred mainly due to the PC 2 values, which in turn were closely related to the chlorophyll absorption band (580 – 690 nm). Based on all of the above, it was concluded that the application of the preparations altered the strawberry fruit development process, resulting in slightly different spectral responses within the chlorophyll-associated wavelength range. After the application of K4, K5 and in part from the K6 preparations, the resulting strawberry

Table 4
The presence of key pathogens of strawberry plantations (*Botrytis cinerea, Colletotrichum* sp., *Phytophthora* sp., and *Verticillium* sp.) indicated by PCR for soil (S), plant (P), and fruit (F). The "+" sign indicates a positive detection of a pathogen in the collected sample. All tests were performed in 3 replications. The "-" sign indicates that the pathogen was not present in all three samples.

Strawberry cultivar	Treatment	PCR *Botrytis cinerea*			PCR *Colletotrichum* sp.			PCR *Phytophthora* sp.			PCR *Verticillium* sp.		
		S	P	F	S	P	F	S	P	F	S	P	F
Honeoye	KI1	+	–	–	–	–	–	+	–	–	++	–	–
	KII1	+++	–	–	–	–	–	+++	–	–	++	–	–
	K2	+	–	–	–	–	–	++	+	–	+	–	–
	K3	+++	–	–	–	–	–	++	+	–	+	–	–
	K4	++	–	–	–	–	–	+++	–	–	+	–	–
	K5	++	–	–	–	–	–	+	–	–	+	–	–
	K6	+++	–	–	–	–	–	–	+	–	+	–	–
Vibrant	KI1	–	–	–	–	–	–	++	–	–	+	–	–
	KII1	++	–	–	–	–	–	+++	–	–	++	–	–
	K2	++	–	–	–	+	–	++	+	–	+	–	–
	K3	++	–	–	–	+	–	+	+	–	+	–	–
	K4	++	–	–	–	–	–	++	–	–	–	–	–
	K5	++	–	–	–	++	–	++	+	–	+	–	–
	K6	+++	–	–	–	+	–	++	+	–	+	–	–
Rumba	KI1	+++	+	–	–	–	–	+++	–	–	+++	–	–
	KII1	+++	–	–	–	–	–	++	–	–	++	–	–
	K2	++	–	–	–	–	–	+	–	–	++	–	–
	K3	+++	–	–	–	–	–	+	+	–	++	–	–
	K4	+	–	–	–	+	–	+	+	–	++	+	–
	K5	+++	–	–	–	+	–	+++	+	–	++	–	–
	K6	+++	–	–	–	–	–	++	–	–	++	–	–

spectra corresponded to fruit at a slightly earlier stage of maturity compared to other treatments and control samples for the Honeoye and Rumba cultivars (Fig. 2a–c). For the Vibrant cultivar, this effect was less pronounced, showing significant differences between the K1I control and the K2, K3, K5 and K6 treatments (but not between K1II).

The tested preparations had a significant effect on the taste attributes of the strawberries. As shown by Carlen and Ançay (2003) among all of the taste attributes the soluble solid content has the highest impact on the consumers' appreciation of the sensory quality of strawberry fruit. The SSC consists mainly of sugars such as glucose, sucrose and fructose which are responsible for the sweet taste of fruit (Tomadoni et al., 2017). Strawberries treated with K4, K5 and K6 preparations showed higher or similar levels of SSC when compared to K1 I samples, while the SSC levels for K1 II, K2 and K3 were lower (Fig. 2a). This effect was mainly attributed to the response of the Honeoye and Vibrant strawberries. According to the model for the assessment of the sensory quality of intact, undamaged and not overripe strawberries about the soluble solid content of the fruit provided by Carlen and Ançay (2003), the SSC levels of the K4, K5 and K6 treated samples belong to the good and excellent quality classes. A different response was observed in the case of the Rumba cv. strawberries, the SSC levels of which decreased after the application of the K2, K3, K4, K5 and K6 preparations, as compared to the K1 I and K1 II control samples (Table 2). In general, the titratable acidity decreases as the fruit matures (Montero et al., 1996). Spayd and Morris (1981) found that the total acidity level increases modestly to a particular maximum level in mature fruit before declining more rapidly in the later stages of ripening. Although they are considered to be taste attributes, studies showed that the sensory quality scores evaluated by consumers had no significant correlation with titratable acidity (Carlen and Ançay, 2003). Nonetheless, acidity showed the opposite trend to SSC, increasing for K2, K3, and K4, and decreasing for the K1 II, K5 and K6 treated strawberries, as compared to the control sample (Fig. 3a). The decrease in acidity may be caused by active metabolism or it may be due to the oxidation of organic acids (Ali et al., 2020).

Taking into account that the level of SSC increases as a result of the conversion of starch into soluble sugars during the rapid ripening and ageing of the fruit (Anjum et al., 2020), one may suppose that the K4, K5, K6 (for Honeoye) and K6 (for Vibrant) preparations accelerated the above-mentioned processes in strawberries. *Bacillus* sp. and *Paenibacillus* sp. bacteria used in consortia produce amylase, which breaks down starch into simple sugars, causing an increase in SSC (Kim et al., 2016). A similar conclusion may be drawn regarding the acidity levels, low levels of which are characteristic of fully matured fruit. However, this is in contrast with the previously discussed spectral data and calculated colour characteristics of the fruit.

The deep dark red colour of strawberry skin is associated with late maturity stages. The changes in strawberry skin colour from green through to white, and up to red, occur due to changes in pigment content. The strawberries owe their red colour to the presence of anthocyanins, the content of which increases during maturation (Sun et al., 2013). Measurements of skin colour using CIE L*a*b colour space showed that after ripening, changes in visual appearance are associated more with luminosity (strawberries become darker) rather than with the hue of strawberries (Sacks and Shaw, 1993). The colour characteristics calculated in this study showed an increase in luminosity (Fig. 1g–1i) for the K4, K5 and K6 treated strawberries of the Honeoye and Rumba cultivars, as compared to other treatments. In the case of the Vibrant cultivar, an increase in the L* values was observed for the K2, K3, K4, K5 and K6 treatments, however, the changes were not statistically significant. At the same time the colour component expressed by values along the a* axis (relative to the green–red opponent colours) showed no significant changes. Therefore, the K4, K5 and K6-treated fruit were brighter than the strawberries treated with other preparations, which is characteristic of earlier maturity stages and additionally, consumers find it to be a more appreciable characteristic.

In relation to other studies concerning the deterioration of the nutritional properties of fruit, polyphenols, anthocyanins and vitamin C were selected as quality indicators of the tested strawberries. Although no statistically significant differences were noted between the polyphenol content and the application of the tested preparations in the Honeoye and Vibrant strawberries, a decrease in the polyphenol content was detected in the Rumba strawberries after treatment with K4, K5 and K6 as compared to K1 I (Table 2). It is possible that these fruits may have experienced increased stress levels, a broken-down cellular structure, and the increased activity of the enzyme polyphenol oxidase produced by microorganisms provided with applied biostimulants (Hussain et al., 2016). Polyphenol oxidase belongs to the group of enzymes that catalyse the oxidation of a wide range of phenolic compounds to quinones. The polymerization of quinones with amino acids or proteins can result in the formation of a brown pigment. The result of these processes is a deterioration in the quality of the food, especially during storage (Adeseko et al., 2021). Moreover, it has been proven that the differences in the content of polyphenols between the cultivars may be relatively large, and the results obtained (121.83–172.64 mg g^{-1} FW) are similar to those of Nowicka et al. (2019) (8.61–208.58 mg 100 g^{-1} FW) and higher than those of Fecka et al. (2021) (average 75.43 mg 100 g^{-1} FW).

Honeoye strawberries also showed no statistically significant differences between the content of anthocyanins and the preparations used in their cultivation (Fig. 2d). On the other hand, the content of anthocyanins in Vibrant fruit increased significantly after treatment with K2, K5 and K6 preparations with the average FW ranging from 35.15 mg 100 g^{-1} FW to 46.54 mg 100 g^{-1} FW (Table 2). Similar results were produced by the Rumba strawberries. The Rumba fruit treated with K3, K5 and K6 showed a slight increase in anthocyanin content as compared to the controls (K I, K1 II) (Table 2). The presented results are consistent with the studies in which the level of the total content of anthocyanins was in the range of 20–60 mg 100 g^{-1} FW depending on the cultivar used and the pelargonidine-3-glucoside level measured ranged from 77 to 90 % (da Silva et al., 2007). It is assumed that the increase in the concentration of anthocyanins may be related to the extent of maturation and the increased level of sugars (Vargas et al., 2006). In almost ripe fruit, the content of pelargonidin-3-glucoside was 3.4–12.4 mg 100 g^{-1} FW, while in fully ripe fruit, the content of pelargonidin-3-glucoside increased by over 3- to 11-fold, to 39–49.6 mg 100 g^{-1} FW (Aaby et al., 2012; Fait et al., 2008).

In the case of vitamin C, the use of a carrier and preparations K2 and K4 (for Vibrant) and K3 (for Rumba) significantly increased the content of vitamin C in comparison with the controls (K1 I, K1 II) by an average of 19 % (for Vibrant) and 16 % (for Rumba). (Table 2). The observed differences in the content of vitamin C could be the result of factors such as the strawberry variety used or the degree of ripeness (Gamboa-Santos et al., 2014). The results obtained are slightly lower than those indicated by Pincemail et al. (2012) (51–185 mg 100 g^{-1} FW) or da Silva Pinto et al. (2008) (65–112 mg 100 g^{-1} FW).

The aforementioned antioxidant compounds are a part of the determined total antioxidant capacity (DPPH) (Du et al., 2009). As the research shows, the DPPH level in the three strawberry cultivars treated with the tested preparations was similar and ranged between 83 and 90 %. In other studies, the DPPH activity ranged from 36 to 93 % (Pinto et al., 2008) and 55–93 % (Mandave et al., 2014) depending on the variety used and the treatment conditions. Nevertheless, certain preparations increased the DPPH level. Only in the case of the Honeoye cultivar, was an increase in the level of DPPH noted in samples treated with K3 and K5 as compared to both controls (K1 I, K1 II) (Table 2). It is assumed that the levels of vitamin C and polyphenols have a great influence on the antioxidant capacity of the fruit (Du et al., 2009). It has also been proven that the antioxidant capacity may depend on the colour of the fruit, which is greatly influenced by the level of antioxidants. In strawberries and other red fruit, the cells in the flesh have many pigments involved in the antioxidant responses. Due to the multiplicity of reaction mechanisms, no test will reflect the total effect of antioxidants on the antioxidant capacity (Du et al., 2009). A higher antioxidant

capacity protects against the effects of oxidative stress. One of the markers of oxidative stress is MDA, the level of which decreased in the Honeoye strawberries treated with K2, K3 and K4 preparations as compared to the controls K1 I and K1 II (Fig. 3d). The MDA content in the Vibrant variety was lower after the use of K3 and K6 with the carrier and with the use of the carrier alone (K1 II) as compared to the K1 I control. The level of MDA used in our research (0.34–2.05 μmol kg^{-1} FW) is higher compared to other studies, where the content of MDA in strawberries is in the range of 0.06–0.18 μmol kg^{-1} FW (Mozafari et al., 2019; Saleem et al., 2021). Higher MDA content determined for strawberries in our research may result from the soil and climatic conditions. It has been proven that the reduction in MDA content may result from the regulation of the level of antioxidant enzymes and thus from a reduction in lipid peroxidation (Ngo and Kim, 2014).

Multiple studies have shown that cell wall modifying enzymes and therefore the metabolism of polysaccharides play an essential role in strawberry fruit ripening (Figueroa et al., 2010; Paniagua et al., 2017; Rosli et al., 2009). Although changes in content were observed for multiple types of polysaccharides, it was demonstrated that changes in the amount of cellulose and hemicellulose do not correlate with strawberry softening (Rosli et al., 2004). In contrast, the closest relationship resulting in softening and quality loss in fruit was observed in changes in the levels of pectic polysaccharides. It has been shown that cell wall modifying enzymes and proteins, including pectinolytic enzymes: α-L-arabinofuranosidase, β-galactosidase, polygalacturonase or pectin methylesterase, are responsible for the degradation of the cell wall components, mostly pectin substances (Brummell and Harpster, 2001). In general, it is an accepted view that cell wall modifications that result in softening involve the solubilization of pectin polysaccharides, depolymerization and the loss of neutral sugars from the pectin side chains (Moya-león et al., 2019). An increased level of enzymatic activity may indicate the degradation of cell wall structures and may also be an indicator of the loss in fruit firmness (Gwanpua et al., 2014). The dismantling of the cell walls also increases the risk of disease development and accelerates fruit rot. Therefore, to quantify the possible effect of the applied treatments on strawberry polysaccharide metabolism, the activities of four major pectinolytic enzymes were measured (Fig. 3).

High B-Gal activity is associated with galactose release and an increase in the total amount of soluble pectin in strawberries (Grosst and Sams, 1984). β-Gal activity increases during fruit development and reaches its highest activity levels in the late stages of strawberry maturity (Paniagua et al., 2016). β-Gal can remove galactose residues in the side chains of rhamnogalacturonans, thereby reducing the interactions between adjacent polysaccharide chains and increasing pectin solubility (Rosli et al., 2004). Taking into account the result that a higher content of water-soluble pectin was found in the softer strawberry varieties (Smith et al., 1998), it may be concluded that a reduction in β-Gal activity may contribute to delaying pectin degradation and solubility (Martinez and Civello, 2008). Although less frequently studied, the AF activity was shown to follow a similar pattern, it increases with fruit maturity (Rosli et al., 2009). In this study, slightly higher activity levels of β-Gal as compared to the untreated samples (K1I) were observed for all treatments except for the K4 preparation. In considering all of the cultivars together, the AF activity did not change concerning the applied treatments (Fig. 3a). None of the tested preparations applied with the carrier decreased the activity of these two enzymes in the Honeoye and Vibrant strawberry varieties (data not shown). In Rumba strawberries, a 26 % decrease in β-Gal activity was detected for samples treated with K3 and K4 preparations. Regardless of the treatment, the activity of β-Gal was less than 1.5–2.4 μmol min^{-1} g^{-1} as detected by Martinez and Civello (2008). Other studies also noted that β-Gal activity was higher than AF activity a similar result to that produced by our analyses (Villarreal et al., 2010).

The remaining two enzymes play a central role in de-estrification (PME) and the following hydrolysis of the 1,4-glycosyl bonds of homogalacturonan, this results in pectin depolymerization (Zhou et al., 2016). It was shown that an increase in the amount of pectin loosely bound to the cell wall correlated with a decrease in strawberry firmness (Posé et al., 2013). Similarly to AF and B-Gal, in this study, the PME levels did not change significantly with the applied treatment (Fig. 3b). Among all of the enzymes tested only PG showed clear differences between treatments, with considerably higher activities for the K1II, K2, K3 and K4 preparations. However, due to the high degree of variability in values, these changes were not statistically significant. In addition to this factor, the observed effects were for the most part influenced by Rumba cv. strawberries, with the remaining two cultivars showing a much less clear response to treatments. It may be concluded that the preparations did not alter the pectic polysaccharide metabolism of the strawberry fruit concerning the activities of the AF, β-Gal, PG and PME enzymes.

Even though the presence of bacterial and fungal microorganisms in the strawberry was observed (Table 3), no fungal and fungal-like pathogens of the genus *Botrytis cinerea, Phytophthora* sp., *Colletotrichum* sp., and *Verticillium* sp. were identified on the strawberry fruit surface (Table 4). This suggests that the bacterial consortia used may prevent the growth of the pathogens tested. The antagonistic activity of the bacteria of the genus *Bacillus subtilis* present in the tested preparations against *Botrytis cinerea* (Samaras et al., 2021), *Verticillium* sp. and *Phytophthora* sp. (Bisutti et al., 2021) and *Colletotrichum* sp. (Kumvinit and Akarapisan, 2016) was confirmed. Similarly, *Paenibacillus polymyxa*, present in K4, K5 and K6, inhibited *Botrytis cinerea* (Helbig, 1986) and *Verticillium* sp. (Rybakova et al., 2017). The preparations used in the experiment belonged to the group of biostimulants used not only for the integrated management of plant diseases but also for the improvement of fruit quality. Apart from bacteria, the preparations contained extracts of nettle, horsetail, calendula, humic acids, yeast leachate, dry bran and rapeseed oil. The multitude of preparation components makes it difficult to determine the mechanisms that take place during the growth and ripening of the fruit. However, in general terms, biostimulants contain a variety of inorganic and organic components, including minerals, polysaccharides, bioactive secondary metabolites, vitamins, and vitamin precursors (MacKinnon et al., 2010; Tirney et al., 2014) that interact synergistically and promote plant growth (Craigie, 2011).

5. Conclusions

Preparations defined as biostimulants are compatible with sustainable agriculture and help to minimize the use of chemical pesticides. Some of the tested preparations (K4, K5 and K6) resulted in the attainment of better quality fruit. It was noted that one of the factors differentiating the effect of the preparations was the variety used. Preparations K4, K5 and K6 in part, slightly delayed the maturation of the strawberries of the Honeoye and Rumba varieties, which resulted in a lighter colour of the strawberry fruit skin, which made them more attractive from the point of view of the consumer. The K6 preparation may be distinguished as the most effective in maintaining high indicators in terms of the postharvest quality of the strawberries. A significant increase in the content of SSC, anthocyanins and vitamin C was observed in the cv. Vibrant strawberries treated with K6. Moreover, Honeoye and Rumba grown in the presence of K6 showed an increase in SSC and anthocyanin content, respectively. The results collected suggest that the K6 preparation influences the taste and health-promoting properties of the tested strawberries. The preparations did not alter the pectic polysaccharide metabolism of the strawberry fruit with respect to the activities of the AF, β-Gal, PG and PME enzymes. Even though the presence of bacteria and fungi in strawberries was observed, the presence of common fungal and fungal-like pathogens belonging to the *Botrytis cinerea, Colletotrichum* sp., *Verticillium* sp. and *Phytophthora* sp. was not detected. Their absence may have been a consequence of the application of the tested carriers and preparations (K1II, K2, K3, K4, K5, K6).

In summary, preparations containing beneficial bacteria, plant extracts, humic acids and others, raise the quality assessment indicators in

strawberries of the Honeoye, Vibrant and Rumba varieties.

CRediT authorship contribution statement

Magdalena Drobek: Writing – original draft, Methodology, Investigation, Formal analysis. **Justyna Cybulska:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Magdalena Frąc:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Piotr Pieczywek:** Writing – review & editing, Visualization, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Giorgia Pertile:** Methodology, Investigation, Formal analysis. **Vadym Chibrikov:** Visualization, Software, Data curation. **Artur Nosalewicz:** Writing – review & editing, Resources, Methodology. **Beata Feledyn-Szewczyk:** Resources, Methodology. **Lidia Sas-Paszt:** Resources. **Artur Zdunek:** Writing – review & editing, Resources, Methodology.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Magdalena Frac reports financial support was provided by National Science Centre Poland. Magdalena Frac has patent #Method for obtaining a bacterial biopreparation and a bacterial biopreparation for maintenance and/or improving soil microbial biodiversity while controlling pathogens: Botrytis sp., Colletotrichum sp., Phytophthora sp., Verticillium sp. in soft fruit cultivation (in Polish), P.445051 pending to Institute of Agrophysics Polish Academy of Sciences. Magdalena Frac has patent #Method for obtaining a microbial fertilizing product and a microbial fertilizing product for conditioning seedlings, maintaining and/or improving the microbiological quality of the soil, while allowing for the control of phytopathogens in the cultivation of soft fruit (in Polish), P.445052 pending to Institute of Agrophysics Polish Academy of Sciences. Magdalena Frac has patent #A method of obtaining a microbiological fertilizing product and a microbiological fertilizing product for maintaining and/or improving the microbiological quality of the soil, allowing at the same time to control the phytopathogens Botrytis sp., Colletotrichum sp., Phytophthora sp., Verticillium sp. in the cultivation of soft fruit (in Polish), P.445053 pending to Institute of Agrophysics Polish Academy of Sciences. Magdalena Frac has patent #Method for obtaining a microbial fertilizing product and a microbial fertilizing product for soil conditioning and improving its biological properties while controlling pathogens Botrytis sp., Colletotrichum sp., Phytophthora sp., Verticillium sp. in soft fruit cultivation (in Polish), P.445054 pending to Institute of Agrophysics Polish Academy of Sciences.

Data availability

Data will be made available on request.

Acknowledgements

This paper was financed by The National Centre for Research and Development within the framework of the NAWA project, contract number PPN/BDE/2019/1/00009/U/00001. This paper was financed by The National Centre for Research and Development within the framework of the BIOSTRATEG project, contract number BIOSTRATEG3/344433/16/NCBR/2018.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.112793.

References

Aaby, K., Mazur, S., Nes, A., Skrede, G., 2012. Phenolic compounds in strawberry (*Fragaria x ananassa* Duch.) fruits: composition in 27 cultivars and changes during ripening. Food Chem. 132, 86–97. https://doi.org/10.1016/j.foodchem.2011.10.037.

Adeseko, C.J., Sanni, D.M., Salawu, S.O., Kade, I.J., Bamidele, S.O., Lawal, O.T., 2021. Biocatalysis and agricultural biotechnology purification and biochemical characterization of polyphenol oxidase of African bush mango (*Irvingia gabonensis*) fruit peel. Biocatal. Agric. Biotechnol. 36, 102119 https://doi.org/10.1016/j.bcab.2021.102119.

Ali, S., Anjum, M.A., Nawaz, A., Naz, S., Ejaz, S., Sardar, H., Saddiq, B., 2020. Tragacanth gum coating modulates oxidative stress and maintains quality of harvested apricot fruits. Int. J. Biol. Macromol. 163, 2439–2447. https://doi.org/10.1016/j.ijbiomac.2020.09.179.

Anjum, M.A., Akram, H., Zaidi, M., Ali, S., 2020. Effect of gum arabic and *Aloe vera* gel based edible coatings in combination with plant extracts on postharvest quality and storability of 'Gola' guava fruits. Sci. Hortic. 271, 109506 https://doi.org/10.1016/j.scienta.2020.109506.

Bisutti, I.L., Pelz, J., Stephan, D., 2021. Influence of antagonistic micro-organisms on the growth of strawberry plants in the presence of *Verticillium dahliae* and *Phytophthora cactorum*. Ecofruit. In: Proceedings of the 15th International Conference on Organic Fruit-Growing. Hohenheim, Germany, pp. 399–403.

Brummell, D.A., Harpster, M.H., 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Mol. Biol. 47, 311–339. https://doi.org/10.1023/A:1010656104304.

Carlen, C., Ançay, A., 2003. Measurement of the sensory quality of strawberries. Acta Hortic. 604, 353–360. https://doi.org/10.17660/ActaHortic.2003.604.37.

Craigie, J.S., 2011. Seaweed extract stimuli in plant science and agriculture. J. Appl. Phycol. 23 (3), 371–393. https://doi.org/10.1007/s10811-010-9560-4.

Cybulska, J., Drobek, M., Panek, J., Cruz-Rubio, J.M., Kurzyna-Szklarek, M., Zdunek, A., Frąc, M., 2022. Changes of pectin structure and microbial community composition in strawberry fruit (Fragaria × ananassa Duch.) during cold storage. Food Chem. 381 https://doi.org/10.1016/j.foodchem.2022.132151.

da Silva, F.L., Escribano-Bailón, M.T., Pérez Alonso, J.J., Rivas-Gonzalo, J.C., Santos-Buelga, C., 2007. Anthocyanin pigments in strawberry. LWT Food Sci. Technol. 40, 374–382. https://doi.org/10.1016/j.lwt.2005.09.018.

da Silva Pinto, M., Lajolo, F.M., Genovese, M.I., 2008. Bioactive compounds and quantification of total ellagic acid in strawberries (*Fragaria x ananassa* Duch.). Food Chem. 107, 1629–1635. https://doi.org/10.1016/j.foodchem.2007.10.038.

Del Buono, D., 2021. Can biostimulants be used to mitigate the effect of anthropogenic climate change on agriculture? It is time to respond. Sci. Total Environ. 751, 141763 https://doi.org/10.1016/j.scitotenv.2020.141763.

Drobek, M., Frąc, M., Zdunek, A., Cybulska, J., 2020. The effect of cultivation method of strawberry (*Fragaria x ananassa* Duch.) cv. honeoye on structure and degradation dynamics of pectin during cold storage. Molecules 25, 4325. https://doi.org/10.3390/molecules25184325.

Du, G., Li, M., Ma, F., Liang, D., 2009. Antioxidant capacity and the relationship with polyphenol and Vitamin C in Actinidia fruits. Food Chem. 113, 557–562. https://doi.org/10.1016/j.foodchem.2008.08.025.

Eggersdorfer, M., Wyss, A., 2018. Carotenoids in human nutrition and health. Arch. Biochem. Biophys. 652, 18–26. https://doi.org/10.1016/j.abb.2018.06.001.

Fait, A., Hanhineva, K., Beleggia, R., Dai, N., Rogachev, I., Nikiforova, V.J., 2008. Reconfiguration of the achene and receptacle metabolic networks during strawberry fruit development. Plant Physiol. 148, 730–750. https://doi.org/10.1104/pp.108.120691.

Fecka, I., Nowicka, A., Kucharska, A.Z., Sokół-Łętowska, A., 2021. The effect of strawberry ripeness on the content of polyphenols, cinnamates, L-ascorbic and carboxylic acids. J. Food Compos. Anal. 95, 103669 https://doi.org/10.1016/j.jfca.2020.103669.

Figueroa, C.R., Rosli, H.G., Civello, P.M., Martínez, G.A., Herrera, R., Moya-León, M.A., 2010. Changes in cell wall polysaccharides and cell wall degrading enzymes during ripening of *Fragaria chiloensis* and *Fragaria* × *ananassa* fruits. Sci. Hortic. 124, 454–462. https://doi.org/10.1016/j.scienta.2010.02.003.

Flores-Félix, J.D., Velázquez, E., García-Fraile, P., González-Andrés, F., Silva, L.R., Rivas, R., 2018. *Rhizobium* and *Phyllobacterium* bacterial inoculants increase bioactive compounds and quality of strawberries cultivated in field conditions. Food Res. Int. 111, 416–422. https://doi.org/10.1016/j.foodres.2018.05.059.

Gamboa-Santos, J., Megías-Pérez, R., Soria, A.C., Olano, A., Montilla, A., Villamiel, M., 2014. Impact of processing conditions on the kinetic of vitamin C degradation and 2-furoylmethyl amino acid formation in dried strawberries. Food Chem. 153, 164–170. https://doi.org/10.1016/j.foodchem.2013.12.004.

Ganugi, P., Martinelli, E., Lucinii, L., 2021. Microbial biostimulants as a sustainable approach to improve the functional quality in plant-based foods: a review. Sci. Total Environ. 140981 https://doi.org/10.1016/j.cofs.2021.05.001.

Gao, Z., Shao, Y., Xuan, G., Wang, Y., Liu, Y., Han, X., 2020. Real-time hyperspectral imaging for the in-field estimation of strawberry ripeness with deep learning. Artif. Intell. Agric. 4, 31–38. https://doi.org/10.1016/j.aiia.2020.04.003.

Grosst, K.C., Sams, C.E., 1984. Changes in cell wall neutral sugar composition during fruit ripening : a species survey. Phytochemistry 23, 2457–2461. https://doi.org/10.1016/S0031-9422(00)84075-3.

Gwanpua, Sunny, G., Buggenhout, S., Van, Verlinden, B.E., Christiaens, S., Shpigelman, A., Vicent, V., Jamsazzadeh, Z., Nicolai, B.M., Hendrickx, M., Geeraerd, A., 2014. Pectin modifications and the role of pectin-degrading enzymes during postharvest softening of Jonagold apples. Food Chem. 158, 283–291. https://doi.org/10.1016/j.foodchem.2014.02.138.

Hangun-Balkir, Y., McKenney, M.L., 2012. Determination of antioxidant activities of berries and resveratrol. Green Chem. Lett. Rev. 5, 147–153. https://doi.org/10.1080/17518253.2011.603756.

Hanson, B.A., 2016. ChemoSpec: Exploratory Chemometrics for Spectroscopy. R Package Version 4.3.17. https://github.com/bryanhanson/ChemoSpec.

Hasing, T.N., Osorio, L.F., Whitaker, V.M., 2013. Within-season stability of strawberry soluble solids content. J. Am. Soc. Hortic. Sci. 138 (3), 190–197. https://doi.org/10.21273/JASHS.138.3.190.

Heil, M., Bostock, R.M., 2002. Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. Ann. Bot. 89, 503–512. https://doi.org/10.1093/aob/mcf076.

Helbig, J., 1986. Biological control of *Botrytis cinerea* pers, ex Fr. in strawberry by *Paenibacillus polymyxa* (Isolate 18191). J. Phytopathol. 149 (5), 265–273. https://doi.org/10.1046/j.1439-0434.2001.00609.x.

Humbal, A., Pathak, B., 2023. Influence of exogenous elicitors on the production of secondary metabolite in plants: a review ("VSI: secondary metabolites"). Plant Stress 8, 100166. https://doi.org/10.1016/j.stress.2023.100166.

Hussain, P.R., Suradkar, P.P., Wani, A.M., Dar, M.A., 2016. Potential of carboxymethyl cellulose and γ-irradiation to maintain quality and control disease of peach fruit. Int. J. Biol. Macromol. 82, 114–126. https://doi.org/10.1016/j.ijbiomac.2015.09.047.

Kim, Y.S., Balaraju, K., Jeon, Y., 2016. Effects of rhizobacteria Paenibacillus polymyxa APEC136 and Bacillus subtilis APEC170 on biocontrol of postharvest pathogens of apple fruits. J. Zhejiang Univ. 17, 931–940.

Kumvinit, A., Akarapisan, A., 2016. Identification of *Colletotrichum acutatum* and screening of antagonistic bacteria isolated from strawberry in Chiang Mai. J. Agric. Technol. 12, 693–706.

Liu, C., Liu, W., Lu, X., Chen, W., Yang, J., Zheng, L., 2014. Application of multispectral imaging to determine quality attributes and ripeness stage in strawberry. PLoS ONE 9. https://doi.org/10.1371/journal.pone.0087818.

Liu, C., Zheng, H., Sheng, K., Liu, W., Zheng, L., 2018. Effects of melatonin treatment on the postharvest quality of strawberry fruit. Postharvest Biol. Technol. 139, 47–55. https://doi.org/10.1016/j.postharvbio.2018.01.016.

Ma, J., Sun, D., Pu, H., 2016. Spectral absorption index in hyperspectral image analysis for predicting moisture contents in pork *longissimus dorsi* muscles. Food Chem. 197, 848–854. https://doi.org/10.1016/j.foodchem.2015.11.023.

MacKinnon, S.L., Hiltz, D., Ugarte, R., Craft, C.A., 2010. Improved methods of analysis for betaines in *Ascophyllum nodosum* and its commercial seaweed extracts. J. Appl. Phycol. 22 (4), 489–494. https://doi.org/10.1007/s10811-009-9483-0.

Mandave, P.C., Pawar, P.K., Ranjekar, P.K., Mantri, N., Kuvalekar, A.A., 2014. Comprehensive evaluation of in vitro antioxidant activity, total phenols and chemical profiles of two commercially important strawberry varieties. Sci. Hortic. 172, 124–134. https://doi.org/10.1016/j.scienta.2014.03.002.

Martinez, G.A., Civello, P.M., 2008. Effect of heat treatments on gene expression and enzyme activities associated to cell wall degradation in strawberry fruit. Postharvest Biol. Technol. 49, 38–45. https://doi.org/10.1016/j.postharvbio.2008.01.013.

Montero, T.M., Moll, E.M., Esteban, R.M., Lopez-Andreu, F.J., 1996. Quality attributes of strawberry during ripening. Sci. Hortic. 65, 239–250. https://doi.org/10.1016/0304-4238(96)00892-8.

Moya-León, M.A., Mattus-Araya, E., Herrera, R., 2019. Molecular events occurring during softening of strawberry fruit. Front. Plant Sci. 10, 615. https://doi.org/10.3389/fpls.2019.00615.

Mozafari, A.A., Ghaderi, N., Havas, F., Dedejani, S., 2019. Comparative investigation of structural relationships among morpho-physiological and biochemical properties of strawberry (*Fragaria* × *ananassa* Duch.) under drought and salinity stresses: a study based on *in vitro* culture. Sci. Hortic. 256, 108601 https://doi.org/10.1016/j.scienta.2019.108601.

Nephali, L., Piater, L.A., Dubery, I.A., Patterson, V., Huyser, J., Burgess, K., Tugizimana, F., 2020. Biostimulants for plant growth and mitigation of abiotic stresses: a metabolomics perspective. Metabolites 10, 1–26. https://doi.org/10.3390/metabo10120505.

Ngo, D.H., Kim, S.K., 2014. Antioxidant Effects of Chitin, Chitosan, and Their Derivatives, Advances in Food and Nutrition Research. Elsevier Inc. https://doi.org/10.1016/B978-0-12-800268-1.00002-0.

Nowicka, A., Kucharska, A.Z., Sokół-Łętowska, A., Fecka, I., 2019. Comparison of polyphenol content and antioxidant capacity of strawberry fruit from 90 cultivars of *Fragaria x ananassa* Duch. Food Chem. 270, 32–46. https://doi.org/10.1016/j.foodchem.2018.07.015.

Nunes, M.C.N., Brecht, J.K., Morais, A.M.M., Sargent, S.A., 1995. Physical and chemical quality characteristics of strawberries after storage are reduced by a short delay to cooling. Postharvest Biol. Technol. 5214, 17–28. https://doi.org/10.1016/0925-5214(94)00048-W.

Paniagua, C., Blanco-Portales, R., Barceló-Muñoz, M., García-Gago, J.A., Waldron, W., Quesada, M.A., Muñoz-Blanco, J., Mercado, J.A., 2016. Antisense down-regulation of the strawberry β -galactosidase gene Fa β Gal4 increases cell wall galactose levels and reduces fruit softening. J. Exp. Bot. 67, 619–631. https://doi.org/10.1093/jxb/erv462.

Paniagua, C., Kirby, A.R., Gunning, A.P., Morris, V.J., Matas, A.J., Quesada, M.A., Mercado, J.A., 2017. Unravelling the nanostructure of strawberry fruit pectins by endo-polygalacturonase digestion and atomic force microscopy. Food Chem. 224, 270–279. https://doi.org/10.1016/j.foodchem.2016.12.049.

Pii, Y., Graf, H., Valentinuzzi, F., Cesco, S., Mimmo, T., 2018. The effects of plant growth-promoting rhizobacteria (PGPR) on the growth and quality of strawberries. Acta Hortic. 1217, 231–238. https://doi.org/10.17660/ActaHortic.2018.1217.29.

Pincemail, J., Kevers, C., Tabart, J., Defraigne, J., Dommes, J., 2012. Cultivars, culture conditions, and harvest time influence phenolic and ascorbic acid contents and antioxidant capacity of strawberry (*Fragaria x ananassa*). J. Food Sci. 77, C205–C210. https://doi.org/10.1111/j.1750-3841.2011.02539.x.

Pinto, M.D.S., Kwon, Y.I., Apostolidis, E., Lajolo, F.M., Genovese, M.I., Shetty, K., 2008. Functionality of bioactive compounds in Brazilian strawberry (*Fragaria x ananassa* Duch.) cultivars: evaluation of hyperglycemia and hypertension potential using *in vitro* models. J. Agric. Food Chem. 56, 4386–4392. https://doi.org/10.1021/jf073275.

PN-A-04019:1998, 1998. Food products. Determination of vitamin C content. Polish Standardization Comm. 1–6.

Posé, S., Paniagua, C., Cifuentes, M., Blanco-Portales, R., Quesada, M.A., 2013. Insights into the effects of polygalacturonase FaPG1 gene silencing on pectin matrix disassembly, enhanced tissue integrity, and firmness in ripe strawberry fruits. J. Exp. Bot. 64, 3803–3815. https://doi.org/10.1093/jxb/ert210.

Pott, D.M., Osorio, S., Vallarino, J.G., 2019. From central to specialized metabolism: an overview of some secondary compounds derived from the primary metabolism for their role in conferring nutritional and organoleptic characteristics to fruit. Front. Plant Sci. 10 https://doi.org/10.3389/fpls.2019.00835.

Preininger, C., Sauer, U., Bejarano, A., Berninger, T., 2018. Concepts and applications of foliar spray for microbial inoculants. Appl. Microbiol. Biotechnol. 102, 7265–7282. https://doi.org/10.1007/s00253-018-9173-4.

Rosli, H.G., Civello, P.M., Martínez, G.A., 2009. α-L-Arabinofuranosidase from strawberry fruit: cloning of three cDNAs, characterization of their expression and analysis of enzymatic activity in cultivars with contrasting firmness. Plant Physiol. Biochem. 47, 272–281. https://doi.org/10.1016/j.plaphy.2008.12.009.

Rosli, H.G., Civello, P.M., Martínez, G.A., 2004. Changes in cell wall composition of three *Fragaria x ananassa* cultivars with different softening rate during ripening. Plant Physiol. Biochem. 42, 823–831. https://doi.org/10.1016/j.plaphy.2004.10.002.

Rouphael, Y., Lucini, L., Miras-Moreno, B., Colla, G., Bonini, P., Cardarelli, M., 2020. Metabolomic responses of maize shoots and roots elicited by combinatorial seed treatments with microbial and non-microbial biostimulants. Front. Microbiol. 11, 1–13. https://doi.org/10.3389/fmicb.2020.00664.

Rybakova, D., Rack-Wetzlinger, U., Cernava, T., Schaefer, A., 2017. Aerial warfare: a volatile dialogue between the plant pathogen *Verticillium longisporum* and its antagonist *Paenibacillus polymyxa*. Front. Plant Sci. 8, 1–10. https://doi.org/10.3389/fpls.2017.01294.

Sacks, E.J., Shaw, D.V., 1993. Color change in fresh strawberry fruit of seven genotypes stored at 0 °C. Hortic. Sci. 28, 209–210.

Saleem, M.S., Anjum, M.A., Naz, S., Ali, S., Hussain, S., Azam, M., Ejaz, S., 2021. Incorporation of ascorbic acid in chitosan-based edible coating improves postharvest quality and storability of strawberry fruits. Int. J. Biol. Macromol. 189, 160–169. https://doi.org/10.1016/j.ijbiomac.2021.08.051.

Samaras, A., Karaoglanidis, G.S., Tzelepis, G., 2021. Insights into the multitrophic interactions between the biocontrol agent *Bacillus subtilis* MBI 600, the pathogen *Botrytis cinerea* and their plant host. Microbiol. Res. 248, 126752 https://doi.org/10.1016/j.micres.2021.126752.

Sangiorgio, D., Cellini, A., Donati, I., Pastore, C., Onofrietti, C., Spinelli, F., 2020. Facing climate change: application of microbial biostimulants to mitigate stress in horticultural crops. Agronomy 10. https://doi.org/10.3390/agronomy10060794.

Sim, K.S., Sri Nurestri, A.M., Norhanom, A.W., 2010. Phenolic content and antioxidant activity of *Pereskia grandifolia* Haw. (Cactaceae) extracts. Pharmacogn. Mag. 6, 248–254. https://doi.org/10.4103/0973-1296.66945.

Smith, D.L., Starrett, D.A., Gross, K.C., 1998. A gene coding for tomato fruit B-Galactosidase II Is expressed during fruit ripening. Plant Physiol. 117, 417–423. https://doi.org/10.1104/pp.117.2.417.

Spayd, S.E., Morris, J., 1981. Physical and chemical characteristics of puree from once-over harvested strawberries. J. Am. Soc. Hortic. Sci. 1, 101–105.

Sun, J., Luo, J., Tian, L., Li, C.L., Xing, Y., Shen, Y.Y., 2013. New evidence for the role of ethylene in strawberry fruit ripening. J. Plant Growth Regul. 461–470. https://doi.org/10.1007/s00344-012-9312-6.

Tirney, M.S., Soler-Vila, A., Rai, D.K., Croft, A.K., Brunton, N.P., Smyth, T.J., 2014. UPLC-MS profiling of low molecular weight phlorotannin polymers in *Ascophyllum nodosum, Pelvetia canaliculata* and *Fucus spiralis*. Metabolomics 10, 524–535. https://doi.org/10.1007/s11306-013-0584-z.

Todeschini, V., Aitlahmidi, N., Mazzucco, E., Marsano, F., Gosetti, F., Robotti, E., Bona, E., Massa, N., Bonneau, L., Marengo, E., Wipf, D., Berta, G., Lingua, G., 2018. Impact of beneficial microorganisms on strawberry growth, fruit production, nutritional quality, and volatilome. Front. Plant Sci. 9, 1–22. https://doi.org/10.3389/fpls.2018.01611.

Tomadoni, B., Cassani, L., Viacava, G., Moreira, M.D.R., Ponce, A., 2017. Effect of ultrasound and storage time on quality attributes of strawberry juice. J. Food Process Eng. 40, 1–8. https://doi.org/10.1111/jfpe.12533.

Vargas, M., Albors, A., Chiralt, A., González-Martínez, C., 2006. Quality of cold-stored strawberries as affected by chitosan-oleic acid edible coatings. Postharvest Biol. Technol. 41, 164–171. https://doi.org/10.1016/j.postharvbio.2006.03.016.

Villarreal, N.M., Bustamante, C.A., Civelloa, P.M., Martinez, G.A., 2010. Effect of ethylene and 1-MCP treatments on strawberry fruit ripening. J. Sci. Food Agric. 90 (4), 683–689. https://doi.org/10.1002/jsfa.3868.

Wei, J., Ma, F., Shi, S., Qi, X., Zhu, X., Yuan, J., 2010. Changes and postharvest regulation of activity and gene expression of enzymes related to cell wall degradation in ripening apple fruit. Postharvest Biol. Technol. 56, 147–154. https://doi.org/10.1016/j.postharvbio.2009.12.003.

Weng, S., Yu, S., Guo, B., Tang, P., Liang, D., 2020. Non-Destructive Detection of Strawberry Quality Using Multi-Features of Hyperspectral Imaging and Multivariate Methods. Sensors 20 (11), 3074. https://doi.org/10.3390/s20113074 (Basel).

Xiaobo, Z., Jiyong, S., Limin, H., Jiewen, Z., Hanpin, M., Zhenwei, C., Yanxiao, L., Holmes, M., 2011. *In vivo* non-invasive detection of chlorophyll distribution in

cucumber (*Cucumis sativus*) leaves by indices based on hyperspectral imaging. Anal. Chim. Acta 706, 105–112. https://doi.org/10.1016/j.aca.2011.08.026.

Xylia, P., Clark, A., Chrysargyris, A., Romanazzi, G., Tzortzakis, N., 2019. Quality and safety attributes on shredded carrots by using *Origanum majorana* and ascorbic acid. Postharvest Biol. Technol. 155, 120–129. https://doi.org/10.1016/j.postharvbio.2019.05.015.

Yan, L., Xiong, C., Qu, H., Liu, C., Chen, W., Zheng, L., 2017. Non-destructive determination and visualisation of insoluble and soluble dietary fibre contents in fresh-cut celeries during storage periods using hyperspectral imaging technique. Food Chem. 228, 249–256. https://doi.org/10.1016/j.foodchem.2017.02.010.

Zhou, H., Li, G., Zhao, X., 2016. Comparative analysis of pectate lyase in relation to softening in strawberry fruits. Can. J. Plant Sci. 96, 604–612. https://doi.org/10.1139/cjps-2015-0323.