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Salicylate Treatment Affects Fruit Quality and Also Alters the Composition of Metabolites in Strawberries

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Abstract: This study evaluated the effect of preharvest treatments with salicylates (salicylic acid (SA), methyl salicylic acid (MeSA) and acetyl salicylic acid (ASA)) on fruit quality parameters and primary and secondary metabolites during ripening at five sampling dates. The results showed that salicylates affect overall fruit quality, and some very desirable and important properties of strawberry fruits were acquired by the treatments, such as a deeper red colour (decreased hue angle), delayed ripening process with maintenance of higher fruit firmness, and higher sugar and ascorbic acid content. HPLC-MS analysis of the phenolic contents showed at almost all sampling dates that treatment with salicylates increased the content of some phenolic groups, the contents of the hydroxycinnamic acids (SA: up to 18%; MeSA: up to 13% increase), flavanols (SA: up to 27%, MeSA: up to 36% and ASA: up to 24% increase), anthocyanins (SA: up to 51%, MeSA: up to 33% and ASA: up to 28% increase) and also flavonol glycosides such as flavones. Total phenolics in fruits also increased-up to 27% with SA and up to 28% with MeSA. In general, better fruit quality and higher metabolite content were obtained with SA.

Keywords: salicylic acid (SA); methyl salicylic acid (MeSA); acetylsalicylic acid (ASA); sugars; organic acids; phenolic compounds; bioactive compounds

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1. Introduction

Strawberry (*Fragaria* × *ananassa*) fruits are appreciated worldwide for their juicy texture, red colour, sweet taste and good source of ascorbic acid, minerals and antioxidative compounds [1,2]. Susceptibility to fungal diseases and short postharvest life are the most undesirable characteristics of strawberry fruit. Scientists are therefore looking for various means of improving the shelf life and quality of strawberry fruit [3].

Phenolic compounds are a diverse group of secondary metabolites in strawberries and have an important role in determining various quality parameters in fruits, such as colour and flavour [4]. Flavonol derivatives, flavanols, hydroxybenzoic acids, hydroxycinnamic acids, ellagic acid derivatives and anthocyanins are the most often analysed phenolic groups in strawberries [5–8]. They also function in plants in growth, reproduction and resistance to pathogens and various environmental stresses [2,6]. For all these reasons, various physical (high and low temperature, ultraviolet and gamma radiation) and chemical triggers, such as chitosan, benzothiadiazole (BTH), ethylene and methyl jasmonate (MeJA), have been explored to increase the content of phenolic compounds in plants [4,9–11].

On the basis of reports by various authors, salicylic acid (SA) and its derivatives could be promising chemicals for improving the above mentioned properties of strawberry fruits [1,3,12,13]. Its use is safe (LD_{50} = 891 mg/kg) and beneficial, also with respect to human health, due to its efficiency in the prevention of cardiovascular diseases [14]. Two SA derivatives are most commonly found, methyl salicylic acid (MeSA) and acetyl salicylic acid (ASA). SA and its derivatives are considered to be plant growth regulators [15]

and signalling molecules, which play an important role in systemic acquired resistance (SAR) [16]. SA plays a key role in stimulation of local defences at the initial site of infection, as well as in distant tissues where there is no infection to induce SAR. On the other hand, MeSA acts as a long-distance SAR signal and occurs even outside the plant, due to its volatile nature [17]. ASA is synthesized from salicylic acid by acetylation with acetic anhydride and it is not defined as a natural plant product [16]. In aqueous solutions, it undergoes spontaneous hydrolysis to SA [18], indicating that exogenously added ASA is rapidly converted to SA. This is also supported by studies on the effects of ASA on plants that are very similar to those of SA. The main difference between aspirin and SA is that ASA can donate an acetyl group in trans acetylation reactions, which inhibit prostaglandin biosynthesis. This is not present in plants, so the significance of this reaction for plants is unknown [16].

Exogenous pre- and post-harvest treatments with salicylates can affect many physiological processes in different plant species. Salicylates can suppress ethylene production and fungal attack [3], induce hypersensitive and systemic acquired resistance [19], increase the activity of some important antioxidant enzymes during storage (e.g., superoxide dismutase, chloramphenicol acetyltransferase and ascorbate peroxidase) [19] and improve fruit quality, such as by increasing ascorbic acid content, total soluble solids and total phenolic content [3,20,21].

Reports are available on the effects of pre-harvest, and especially post-harvest treatments with salicylates, on fruit quality characteristics of many different plant species [22–24]. However, we observed that the effects of salicylate treatments on different secondary metabolites, especially on individual phenolic compounds, are poorly defined. The main objective of our investigation was to determine the influence of different pre-harvest salicylate treatments on the main fruit quality parameters (fruit firmness, fruit weight, fruit colour, ascorbic acid and sugar content) and on the content of individual and total phenolic compounds in strawberry fruit by HPLC connected with mass spectrometry.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Material

The open field experiment was conducted in 2018 at the experimental station located at Brdo pri Lukovici (latitude, 46°10′ N; longitude, 14°41′ E), which is owned by the Agricultural Institute of Slovenia. Frigo plants of the 'Clery' cultivar were planted on 26 July 2016 in two rows on growth ridges covered with black polyethylene foil at a growth distance of 0.25 m × 0.25 m. The growth ridges were equipped with an irrigation system. Strawberry production was regulated according to the practice of integrated production. The experiment included four different treatments, which differed only in terms of spraying with different 1 mM solutions of salicylic acid (SA), 1 mM methyl salicylic acid (MeSA), 1 mM acetyl salicylic acid (ASA) and water (Control, C). Sprayings were done with a backpack sprayer. Each treatment was repeated in five blocks (1, 2, 3, 4, 5) and each block included 10 plants. Sampling dates were selected according to the ripening of the strawberries. Fully ripe strawberries were collected at the five sampling dates during the entire ripening season to evaluate outer and inner fruit quality parameters. The first sampling date was before any spraying–T0. Spraying with water (control), 1 mM SA, 1 mM MeSA and 1 mM ASA were on the same day (21st May). Thereafter, the strawberries were sampled four more times, on May 22 (24 h after treatment–24 h), May 24 (72 h after treatment–72 h), May 28 (7 days after treatment–7 days) and May 31 (10 days after treatment–10 days). For primary and secondary metabolite analysis, eight strawberries per treatment and block were sampled at each sampling date, frozen in liquid nitrogen and stored at −20 °C.

2.1.2. Chemicals

For determination of sugars, sucrose, fructose and glucose standards from Fluka Chemie (Buchs, Switzerland) were used. Organic acids were determined with the use of

citric, malic, ascorbic and fumaric acid standards from Fluka Chemie and shikimic acid from Sigma Aldrich (St. Louis, MO, USA). Flavanols were quantified according to the standard curves of the following standards: procyanidin B1, catechin (Fluka Chemie), flavonols according to quercetin-3-*O*-galactoside, quercetin-3-*O*-rutinoside, kaempferol-3-*O*-glucoside from Fluka Chemie and isorhamnetin-3-*O*-glucoside from Extrasynthèse, flavones according to apigenin-7-*O*-gucoside (Fluka Chemie), flavanones according to eriodictyol-7-*O*-glucoside and naringenin (Sigma-Aldrich), hydroxycinnamic acids according to chlorogenic acid and caffeic acid (Sigma-Aldrich), ellagitannins according to ellagic acid (Sigma-Aldrich) and anthocyanins according to cyanidin-3-glucoside, pelargonidin-3-glucoside (Sigma-Aldrich).

Methanol for the extraction of phenolic compounds was purchased from Sigma Aldrich. Mobile phases for phenolics analysis with HPLC-MS were made with acetonitrile and formic acid from Sigma Aldrich. Water for the mobile phase for sugars and organic acids (4 mM sulphuric acid) was double distilled and purified with a MilliQ system (Millipore, Bedford, MA, USA).

2.1.3. Fruit Quality Parameters Measurements

To evaluate the effects of preharvest treatments with salicylates on strawberry fruit quality characteristics, fruit weight (g), fruit firmness (kg/cm^2), soluble solids content (°Brix) and colour parameters (lightness-*L**, chroma-*C* and hue angle-h° parameters) were measured, on three randomly selected strawberry fruits for each block (1, 2, 3, 4, 5) and each treatment (C, SA, MeSA, ASA) immediately after harvest at each sampling date (T0, 24 h, 72 h, 7 days and 10 days after spraying with different salicylates). Each strawberry fruit was first weighed on a precision balance (Model ALS 120-4N, Kern, Balingen, Germany), with a confidence level of 0.001 g. The firmness of the fruit was determined by measurements with a penetrometer (T.R. Turoni srl, Forlì, Italy) with a 3 mm tip. A digital refractometer (WM-7; Atago; Tokyo, Japan) was used to measure soluble solids content in strawberry fruits. Colour measurements on strawberries were made with a portable colorimeter (CR-200 b Chroma; Minolta, Osaka, Japan).

2.1.4. Sugars, Organic Acids and Ascorbic Acid Analysis

Strawberries were randomly selected for each treatment (C, SA, MeSA, ASA) in each block (1, 2, 3, 4, 5) on each sampling date (T0, 24 h, 72 h, 7 days and 10 days after SAL spraying). Sample extraction for sugars and organic acids quantification was carried out by the protocol reported by Mikulic-Petkovsek et al. [8]: we first mashed the samples to a homogenized material in a previously chilled mortar and then weighed 5 g of sample in a centrifuge tube and poured over 26 mL bidistilled water. For ascorbic acids extraction, 11 mL of 2% solution of *m*-phosphoric acid was poured over 5 g of homogenized plant material. The samples for both types of analysis were stirred continuously for half an hour at 23 °C. The samples were then centrifuged at 8000 rpm for 7 min at 10 °C (Eppendorf Centrifuge 5819 R, Hamburg, Germany) and filtered through cellulose ester filters (0.20 µm; Macherey-Nagel, Düren, Germany) into glass vials, and the extracts were analysed using a high-pressure liquid chromatography (HPLC) system (Thermo Scientific, San Jose, CA, USA).

The HPLC conditions and equipment for all type of analysis are shown in Table S1. Identification of sugars (fructose, sucrose, glucose) and organic acids (citric, malic, ascorbic, fumaric acid and shikimic acid) present in strawberries was performed using external standards. The results were then calculated and expressed as g of sugar or organic acid per kg fresh weight (FW). Since we were interested in the content of ascorbic acid in the recommended serving of strawberries (about five fruits), we expressed the content in mg/100 g of FW.

2.1.5. Total and Individual Phenolic Compounds Analysis

Sampling for phenolic compounds analysis was performed on five sampling dates (T0, 24 h, 72 h, 7 days and 10 days after SAL spraying). Three randomly chosen fruits for each treatment (C, SA, MeSA, ASA) in each block (1, 2, 3, 4, 5) were immediately extracted. Five grams of previously homogenized sample was weighed in a centrifuge tube and 5 mL of extraction solution was poured over (mixture of 3% formic acid in 80% methanol) as previously reported by Mikulic-Petkovsek et al. [8]. Extraction took place in a cooled ultrasonic bath (Sonis 3, Iskra PIO, Slovenia) at 2 °C for one hour. The samples were then centrifuged at 8000× *g* for 7 min at 4 °C (5819 R; Eppendorf, Germany), and filtered into vials using 0.20 µm polyamide filters (Chromafil AO-20/25 polyamide filters; Macherey-Nagel, Germany).

Quantification of the individual phenolic compounds in strawberries was carried out using the Accela HPLC system (Thermo Scientific; San Jose, CA, USA) with the DAD detector adjusted to different wavelengths related to the different phenolic groups present in the plant material. Flavanols, hydroxycinnamic acids and some ellagic acid derivatives were analysed at 280 nm, flavonols, flavanones, flavones and some ellagic acid derivatives at 350 nm and anthocyanins at 530 nm. HPLC conditions and equipment for phenolic compound analysis are presented in Table S1. Identification of all presented phenolic compounds in the plant material was made by comparing their retention times with standards and using mass spectrometry (LTQ XLTM Linear Ion Trap mass spectrometer; Thermo Scientific) with electrospray ionisation. The scans were from *m/z* 115 to 1500, operating in negative ion mode for flavanols, flavonols, flavanones, flavones, hydroxycinnamic acids, ellagic acid derivatives and in positive ion mode for anthocyanins. MS conditions are presented in Table S1 and were as reported in Mikulič-Petkovšek et al. [7], with some modifications. Extracts were eluted according to the gradient method presented in Table S1. Spectral data were processed by Excalibur software (Thermo Fischer Scientific, Waltham, USA). The contents of phenolic compounds were calculated from peak areas of the sample and the corresponding standards and expressed in mg/kg FW. Total analysed phenolics (TAP) are presented as the sum of all analysed phenolic compounds in strawberry fruits.

2.1.6. Statistical Analysis

R-commander (R Formation for Statistical Computing, Auckland, New Zealand) statistical software was used to analyse the data. The individual effects of the salicylates treatments on the fruit quality characteristics, primary metabolites and on the secondary metabolites in the strawberry fruits was subjected to one-way analysis of variance (ANOVA). Significant differences among the treatments were calculated with multiple comparisons of means with the Tukey test and Tukey contrasts ($p < 0.05$).

3. Results

3.1. Effects of Salicylates on Fruit Colour, Firmness, Fruit Weight and Soluble Solids

The effects of different salicylic acid derivatives (salicylic acid (SA), methyl salicylic acid (MeSA) and acetyl salicylic acid (ASA)) on colour (lightness-*L**, chroma-C and hue angle-h° parameters), fruit weight (g), fruit firmness (kg/cm^2) and soluble solids content (°Brix) of strawberry fruits were investigated (Table 1). The date collected showed that the fruit firmness and hue angle of the strawberry fruits was significantly influenced by salicylate treatments (Table 1). Thus, fruit firmness (Table 1) was significantly higher ($p < 0.05$) in most strawberry fruits treated with SA, ASA and MeSA than in controls at all sampling dates except 7 days after salicylate treatments. On the sampling date 72 h after treatments, clearer differences among salicylates occurred. The firmness of ASA treated strawberries was 19.7% lower and 39.4% lower for MeSA treated fruits than SA treated fruits. The hue angle (h*) of the strawberries was significantly ($p < 0.05$) lower in all treated fruits than in control ones (Table 1), which showed a deep red colour as a consequence of treatments. When sampled 72 h after treatment with salicylates, a significantly lower ($p < 0.05$) hue angle was observed in fruits treated with SA compared to all other treatments.

On the other hand, treatments with salicylates showed no significant effects on fruit weight, soluble solids content, *L** and *C* parameters (Table 1).

Table 1. Effects of different salicylates (SA, MeSA, ASA) on colour (lightness-*L**, chroma-*C* and hue angle-h° parameters), fruit weight (g), fruit firmness (kg/cm^2) and soluble solids content (°Brix) of strawberry fruits at five different sampling dates (T0, 24 h, 72 h, 7 days and 10 days after treatments with salicylates).

	Treatment	*L**	C	h°	Fruit Firmness (kg/cm^2)	Soluble Solids (°Brix)	Fruit Weight (g)
T0	C	33.54 ± 0.52 a	40.03 ± 0.94 a	31.15 ± 0.63 a	0.79 ± 0.09 a	7.89 ± 0.30 a	13.21 ± 0.75 a
	SA	33.93 ± 0.64 a	41.47 ± 0.87 a	31.24 ± 0.58 a	0.88 ± 0.09 a	7.18 ± 0.23 a	12.03 ± 0.86 a
	MeSA	33.16 ± 0.55 a	39.17 ± 1.09 a	31.11 ± 0.98 a	0.98 ± 0.11 a	7.43 ± 0.28 a	11.55 ± 0.45 a
	ASA	33.14 ± 0.45 a	41.56 ± 0.93 a	31.97 ± 0.56 a	0.98 ± 0.09 a	7.99 ± 0.24 a	12.86 ± 0.51 a
24 h	C	32.74 ± 2.18 a	42.25 ± 1.21 a	31.46 ± 0.27 b	0.73 ± 0.04 a	7.57 ± 0.19 a	13.05 ± 0.74 a
	SA	34.37 ± 0.70 a	41.23 ± 1.00 a	27.45 ± 0.69 a	1.07 ± 0.08 b	8.97 ± 0.23 b	13.78 ± 0.66 a
	MeSA	33.42 ± 0.63 a	39.27 ± 1.26 a	28.77 ± 0.52 a	0.94 ± 0.04 b	7.91 ± 0.26 a	13.64 ± 0.80 a
	ASA	33.71 ± 0.54 a	40.92 ± 1.69 a	28.02 ± 0.76 a	0.88 ± 0.04 ab	7.88 ± 0.32 a	14.07 ± 0.91 a
72 h	C	34.90 ± 0.6 ab	42.00 ± 0.94 a	32.69 ± 0.30 d	0.49 ± 0.04 ab	8.66 ± 0.34 a	12.45 ± 0.72 a
	SA	34.67 ± 0.53 ab	42.81 ± 0.78 a	27.15 ± 0.29 a	0.66 ± 0.03 c	9.07 ± 0.21 a	12.99 ± 0.75 a
	MeSA	33.20 ± 0.58 a	40.66 ± 0.82 a	28.80 ± 0.44 b	0.40 ± 0.03 a	8.53 ± 0.35 a	13.08 ± 0.69 a
	ASA	35.31 ± 0.40 b	42.43 ± 0.76 a	30.46 ± 0.54 c	0.53 ± 0.03 bc	8.59 ± 0.34 a	15.08 ± 0.9 a
7 days	C	33.33 ± 0.49 a	37.43 ± 1.20 a	29.32 ± 0.37 b	0.45 ± 0.03 a	8.97 ± 0.47 a	8.54 ± 0.74 ab
	SA	34.09 ± 0.67 a	38.58 ± 1.12 a	27.25 ± 0.52 a	0.34 ± 0.04 a	10.49 ± 0.44 a	8.60 ± 0.38 b
	MeSA	32.63 ± 0.49 a	35.60 ± 1.05 a	26.28 ± 0.55 a	0.33 ± 0.03 a	8.82 ± 0.50 a	7.48 ± 0.50 a
	ASA	32.63 ± 0.71 a	36.80 ± 1.40 a	26.81 ± 0.70 a	0.33 ± 0.03 a	8.90 ± 0.36 a	8.60 ± 0.66 ab
10 day	C	32.97 ± 0.62 a	36.13 ± 1.22 a	29.65 ± 0.39 b	0.25 ± 0.02 a	8.57 ± 0.47 a	7.23 ± 0.48 a
	SA	33.21 ± 0.67 a	36.81 ± 1.05 a	26.69 ± 0.55 a	0.39 ± 0.04 b	9.13 ± 0.48 a	6.99 ± 0.37 a
	MeSA	32.30 ± 0.85 a	34.15 ± 1.66 a	26.83 ± 0.51 a	0.45 ± 0.04 b	8.57 ± 0.38 a	6.20 ± 0.31 a
	ASA	32.70 ± 0.46 a	36.03 ± 0.95 a	27.19 ± 0.27 a	0.32 ± 0.05 ab	9.17 ± 0.47 a	7.16 ± 0.39 a

Abbreviations: SA—salicylic acid, MeSA—methyl salicylic acid, ASA—acetyl salicylic acid, C—control, h—hours. Data are presented as average value ± standard error (n = 15). Different letters in a table denote significant differences among treatments (C, SA, MeSA, ASA) at each time point, calculated by the Tukey test ($p < 0.05$).

3.2. Effects of Salicylates on Primary Metabolites in Strawberry Fruits

The contents of individual sugars, organic acids and ascorbic acid (Table 2) were determined in strawberry fruits treated with salicylates (SA, ASA, MeSA) at five sampling points (T0, 24 h, 72 h 7 days and 10 days after treatment). Glucose and fructose were the predominant sugars at all sampling dates and accounted for 78–93% of all identified sugars in strawberry fruits (Table 2). Except for the sampling date 7 days after treatment, all salicylates had a significant effect on the increase in sugars in the strawberries ($p < 0.05$). Citric acid represented 82–93% of all organic acids identified. Malic, fumaric and shikimic (in traces) acid also appeared in smaller amounts (Table 2). Treatments with salicylates generally had no effect on the acid content. Only at two sampling dates (24 h and 72 h after treatments) did strawberries treated with SA contain on average 22% (24 h) and 16% (72 h) less total organic acids than the control. The results showed that ascorbic acid in the strawberry fruits was affected by SA treatment (Table 2) at all sampling dates except 7 days after treatment compared to the control. On average, an increase in ascorbic acid content of up to 17% was observed with SA treatment. Other salicylates had no significant effect, although a slight increase was observed compared to the control.

Table 2. Content of Sugars (g/kg FW), Organic Acids (g/kg FW) and Ascorbic Acid (mg/100 g FW) in Salicylic Acid (SA), Methyl Salicylic Acid (MeSA) and Acetyl Salicylic Acid (ASA) Treated Strawberries at Different Time Points (T0, 24 h, 72 h, 7 days and 10 days after treatments with salicylates).

	Treatment	Sucrose	Glucose	Fructose	Total Sugars [a]	Citric	Malic	Fumaric	Total Acids [b]	Ascorbic Acid
T0	C	6.70 ± 1.09 a	21.38 ± 1.23 a	21.76 ± 1.25 a	49.81 ± 3.53 a	6.77 ± 0.26 a	0.53 ± 0.03 a	0.40 ± 0.02 a	7.70 ± 0.24 a	38.34 ± 1.65 a
	SA	5.45 ± 0.92 a	21.15 ± 1.01 a	21.49 ± 1.03 a	48.1 ± 2.85 a	6.76 ± 0.62 a	0.52 ± 0.03 a	0.39 ± 0.02 a	7.67 ± 0.62 a	40.22 ± 2.05 a
	MeSA	6.47 ± 0.75 a	22.59 ± 1.22 a	22.95 ± 1.24 a	52.01 ± 3.06 a	6.46 ± 0.63 a	0.53 ± 0.02 a	0.40 ± 0.02 a	7.41 ± 0.63 a	42.55 ± 4.58 a
	ASA	7.39 ± 0.84 a	22.58 ± 0.84 a	22.94 ± 0.85 a	52.92 ± 2.43 a	7.30 ± 0.33 a	0.53 ± 0.04 a	0.40 ± 0.03 a	8.24 ± 0.36 a	41.57 ± 1.96 a
24 h	C	5.11 ± 0.58 a	16.64 ± 0.55 a	18.17 ± 0.58 a	39.91 ± 1.11 a	5.13 ± 0.25 a	0.39 ± 0.04 a	0.35 ± 0.02 a	7.51 ± 0.35 b	33.97 ± 1.05 a
	SA	6.71 ± 0.21 a	21.27 ± 0.80 b	22.41 ± 0.45 c	50.39 ± 1.05 b	6.61 ± 0.32 b	0.51 ± 0.02 b	0.39 ± 0.02 a	5.88 ± 0.26 a	41.06 ± 1.32 b
	MeSA	6.77 ± 0.67 a	19.63 ± 0.32 ab	19.95 ± 0.33 ab	46.34 ± 1.20 ab	6.00 ± 0.25 ab	0.47 ± 0.03 ab	0.36 ± 0.02 a	6.84 ± 0.28 ab	38.03 ± 0.84 ab
	ASA	7.53 ± 1.28 a	20.50 ± 1.21 b	21.64 ± 0.53 bc	49.67 ± 2.96 b	6.41 ± 0.15 b	0.52 ± 0.03 b	0.39 ± 0.02 a	7.31 ± 0.16 b	37.86 ± 0.92 ab
72 h	C	8.55 ± 0.52 a	22.93 ± 0.46 a	22.52 ± 0.36 a	54.00 ± 1.02 a	5.95 ± 0.26 ab	0.48 ± 0.03 a	0.36 ± 0.02 a	8.12 ± 0.27 b	37.69 ± 0.91 a
	SA	10.86 ± 0.46 bc	26.03 ± 0.20 b	26.46 ± 0.21 c	63.35 ± 0.59 b	7.22 ± 0.27 b	0.51 ± 0.02 a	0.38 ± 0.02 a	6.79 ± 0.30 a	43.83 ± 1.52 b
	MeSA	9.58 ± 0.57 ab	24.79 ± 0.74 ab	24.19 ± 0.74 ab	58.57 ± 1.80 ab	6.13 ± 0.36 ab	0.48 ± 0.02 a	0.36 ± 0.01 a	6.97 ± 0.36 ab	38.40 ± 1.46 ab
	ASA	12.16 ± 0.69 c	24.32 ± 0.54 ab	25.10 ± 0.47 bc	61.58 ± 1.53 b	5.66 ± 0.37 a	0.52 ± 0.03 a	0.39 ± 0.02 a	6.57 ± 0.35 a	38.49 ± 1.88 ab
7 days	C	6.49 ± 0.51 a	22.11 ± 1.02 a	21.86 ± 2.25 a	50.47 ± 2.44 a	5.88 ± 0.44 a	0.4 ± 0.02 a	0.30 ± 0.02 a	6.59 ± 0.45 a	43.44 ± 3.01 a
	SA	10.61 ± 0.63 b	25.42 ± 1.59 a	25.82 ± 1.62 a	61.85 ± 3.01 a	5.46 ± 0.49 a	0.46 ± 0.04 a	0.34 ± 0.03 a	6.26 ± 0.55 a	42.99 ± 2.21 a
	MeSA	10.55 ± 0.86 b	23.69 ± 2.00 a	24.08 ± 2.03 a	58.32 ± 4.20 a	5.79 ± 0.33 a	0.43 ± 0.03 a	0.32 ± 0.02 a	6.54 ± 0.33 a	42.80 ± 1.38 a
	ASA	9.06 ± 0.47 b	23.36 ± 1.62 a	23.74 ± 1.64 a	56.16 ± 3.57 a	4.94 ± 0.40 a	0.42 ± 0.02 a	0.32 ± 0.01 a	5.68 ± 0.42 a	39.09 ± 2.11 a
10 days	C	6.97 ± 0.55 a	19.10 ± 0.78 a	23.70 ± 3.4 a	49.08 ± 1.03 ab	5.23 ± 0.29 a	0.45 ± 0.04 a	0.34 ± 0.03 a	6.02 ± 0.31 a	41.16 ± 1.60 a
	SA	7.26 ± 0.97 a	23.57 ± 0.89 b	24.13 ± 0.94 a	54.96 ± 2.27 bc	6.44 ± 0.39 a	0.29 ± 0.02 a	0.29 ± 0.02 a	7.11 ± 0.39 a	49.44 ± 1.19 b
	MeSA	9.09 ± 0.48 a	24.79 ± 0.42 b	25.20 ± 0.43 a	59.08 ± 1.03 c	6.08 ± 0.50 a	0.31 ± 0.02 a	0.31 ± 0.02 a	6.82 ± 0.48 a	49.38 ± 2.78 b
	ASA	7.34 ± 0.34 a	18.61 ± 0.67 a	18.10 ± 0.66 a	44.06 ± 0.94 a	4.97 ± 0.35 a	0.29 ± 0.01 a	0.29 ± 0.01 a	5.66 ± 0.36 a	40.13 ± 2.17 a

Abbreviations: SA—salicylic acid, MeSA—methyl salicylic acid, ASA—acetyl salicylic acid, C—control, h-hours. Data are presented as average value ± standard error (n = 15). Different letters in a table denote significant differences in sugars, organic acids and ascorbic acid contents among treatments (C, SA, MeSA, ASA) at each time point, calculated by the Tukey test ($p < 0.05$). [a] Sum of all identified sugars. [b] Sum of all identified organic acids.

3.3. Effect of Salicylates on Secondary Metabolites in Strawberry Fruits

Thirty-four different phenolic compounds (Table 3; Table S2–S8) were identified in the strawberry fruits, which are arranged in 7 different phenolic groups: hydroxycinnamic acid derivatives, flavanols, flavonols, flavanones, flavones, ellagic acid derivatives and anthocyanins. Figure 1 presents phenolic chromatograms of strawberry fruit recorded at (A) 280 nm, (B) 350 nm and (C) 530 nm. Peak numbers are described in Table 3.

Table 3. Identification of phenolic compounds in strawberry fruit of cv. 'Clery' in negative or positive ionization with HPLC-MS and MS^2.

Peak No	Identified Phenolic Compounds	$[M-H]^-$ (*m*/*z*)	MS^2 (*m*/*z*)	Phenolic Group
1	bis HHDP hexose 1	783	481,301	EAD
2	Cyanidin-3-glucoside	449	287	ANT
3	bis HHDP hexose 2	783	481,301	EAD
4	Procyanidin dimer 1	577	425,407,289	FLA
5	Pelargonidin-3-glucoside	433	271	ANT
6	Pelargonidin-3-rutinoside	579	271	ANT
7	Procyanidin dimer 2	577	425,407,289	FLA
8	Chlorogenic acid	353	191,179	HCAD
8	Catechin	289	245	FLA
9	p-coumaric acid hexoside	325	163,119	HCAD
10	Caffeic acid derivative	335	179,135	HCAD
11	Cyanidin-3-malonylglucoside	535	287	ANT
12	Pelargonidin-3-malonylglucoside	519	271	ANT
13	Cinamoyl hexoside	355	309,207,147	HCAD
14	Apigenin rhamnoside	415	269	FVN
15	Apigenin acetyl hexoside 1	473	269	FVN
16	Apigenin acetyl hexoside 2	473	269	FVN
16	Eriodictyol acetyl hexoside	491	287	FLV
17	Ellagic acid pentoside 1	433	301	EAD
18	Ellagic acid hexoside	463	301	EAD
19	Naringenin hexoside	433	271	FLV
20	Ellagic acid rhamnoside	447	301	EAD
21	Ellagic acid deoxy hexoside	447	301	EAD
22	Ellagic acid pentoside 2	433	301	EAD
23	Quercetin-pentose hexoside	595	301	FLO
24	Quercetin-3-rutinoside	609	301	FLO
25	Quercetin-3-glucoside	463	301	FLO
26	Kaempferol-3-rutinoside	593	285	FLO
27	Quercetin-3-glucuronide	477	301	FLO
28	Kaempferol-3-glucoside	447	285	FLO
29	Isorhamnetin hexoside	477	315	FLO
30	Kaempferol-3-glucuronide	461	285	FLO
31	Kaempferol acetyl hexoside	489	285	FLO
32	Kaempferol-3-coumaroyl glucoside	593	285	FLO

M^+ (*m*/*z*) anthocyanins were obtained in the positive ion mode, other phenolics in the negative ion mode. Legend: HCAD-hydroxycinnamic acid derivatives, FLA-flavanols, FLO-flavonols, FVN-flavones, FLV-flavanones, EAD-ellagic acid derivatives, ANT-anthocyanins.

The largest share of total identified phenolic compounds (TAP) (Table 4) in the strawberry fruits was flavanols (30–33% TAP) and anthocyanins (33–34% TAP). Treatments with SA, MeSA and ASA resulted in a significant ($p < 0.05$) increase in TAP compared to the control, although no significant differences were observed at the last two sampling dates. However, the trend towards an increase in TAP content in strawberries was still evident compared to the control. In general, better results were obtained with the treatments SA and MeSA, which caused an increase of up to 27% (SA) and up to 28% (MeSA) in TAP.

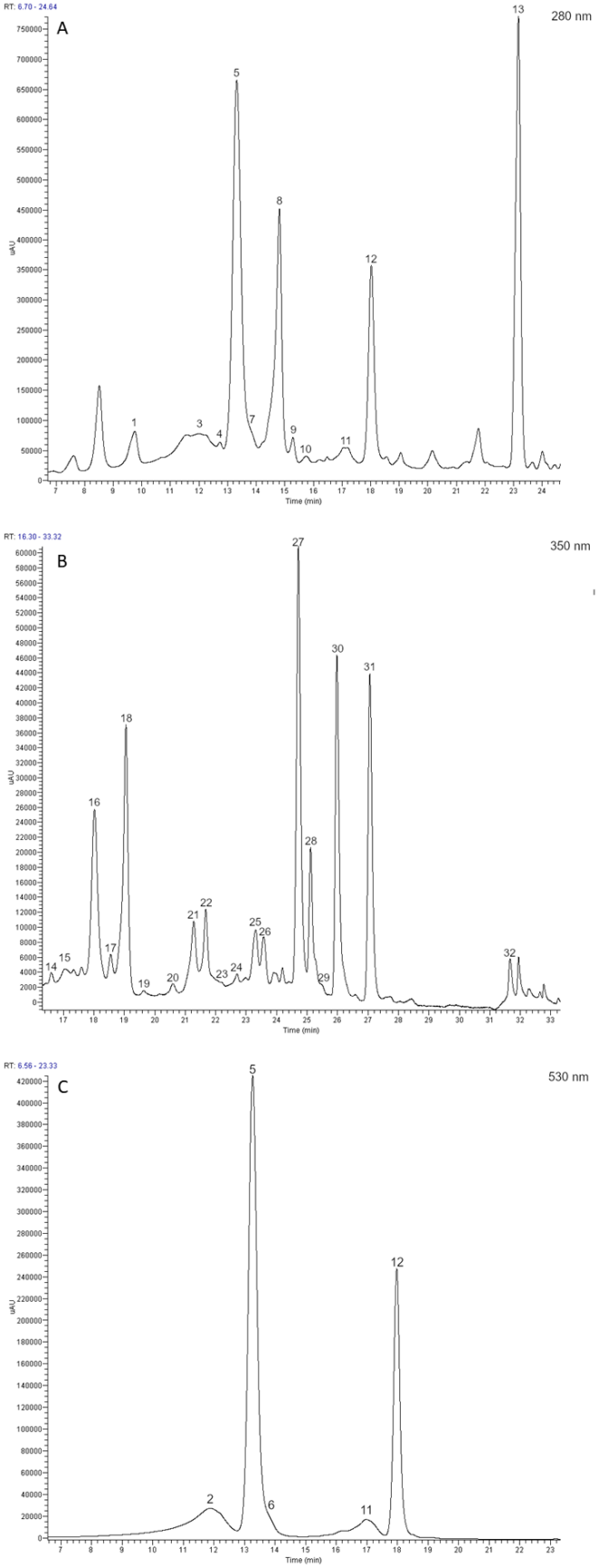

Figure 1. Phenolic chromatograms of strawberry fruit recorded at (**A**) 280 nm, (**B**) 350 nm and (**C**) 530 nm. Peak numbers are described in Table 3.

Table 4. Content of Total Identified Phenolic Groups (mg/kg FW) in Salicylic Acid (SA), Methyl Salicylic Acid (MeSA) and Acetyl Salicylic Acid (ASA) Treated Strawberries at Different Time Points (T0, 24 h, 72 h, 7 days and 10 days after treatments with salicylates) [a].

	Treatment	Hydroxycinn-Amic Acid Derivatives [b]	Flavonols [a]	Flavanols [a]	Flavanones [a]	Flavones [a]	Ellagic Acid Derivatives [a]	Anthocyanins [a]	Total Phenolics [b]
T0	C	174.39 ± 6.72 a	11.19 ± 1.44 a	354.81 ± 10.20 a	1.14 ± 0.09 a	114.48 ± 1.80 a	116.37 ± 7.35 a	386.73 ± 8.37 a	1169.52 ± 11.37 a
	SA	184.80 ± 12.15 a	10.23 ± 0.93 a	349.80 ± 2.28 a	1.08 ± 0.21 a	106.53 ± 2.79 a	117.54 ± 8.43 a	392.07 ± 13.62 a	1171.11 ± 18.90 a
	MeSA	185.55 ± 8.88 a	9.81 ± 1.83 a	345.66 ± 20.61 a	1.23 ± 0.18 a	109.80 ± 2.46 a	118.65 ± 6.00 a	406.50 ± 16.20 a	1170.20 ± 41.31 a
	ASA	180.72 ± 4.26 a	10.74 ± 2.31 a	363.15 ± 16.74 a	0.99 ± 0.15 a	112.53 ± 4.74 a	107.04 ± 7.98 a	373.50 ± 12.09 a	1160.49 ± 32.4 a
24 h	C	184.59 ± 1.74 a	6.21 ± 0.33 a	310.47 ± 10.53 a	1.26 ± 0.12 a	110.55 ± 2.67 a	115.68 ± 9.18 a	298.80 ± 2.37 a	1040.10 ± 18.72 a
	SA	215.76 ± 1.77 c	11.70 ± 0.87 b	396.90 ± 8.91 c	1.05 ± 0.18 a	129.54 ± 2.73 b	111.81 ± 10.83 a	450.06 ± 7.59 d	1323.63 ± 17.07 c
	MeSA	197.97 ± 2.55 b	10.83 ± 1.50 b	373.02 ± 5.40 bc	1.32 ± 0.12 a	129.63 ± 5.34 b	115.98 ± 7.74 a	333.60 ± 6.42 b	1172.31 ± 17.49 b
	ASA	185.22 ± 1.62 a	10.56 ± 0.57 b	335.25 ± 17.16 ab	1.20 ± 0.18 a	129.54 ± 3.93 b	111.39 ± 14.88 a	372.03 ± 9.54 c	1155.48 ± 30.84 b
72 h	C	220.53 ± 6.51 a	6.39 ± 0.99 a	382.71 ± 17.01 a	1.08 ± 0.12 a	108.15 ± 7.62 a	126.36 ± 11.76 a	352.83 ± 8.16 a	1204.26 ± 64.41 a
	SA	253.17 ± 7.92 b	13.86 ± 0.66 b	482.85 ± 11.55 b	1.14 ± 0.15 a	116.40 ± 5.64 a	136.44 ± 3.36 a	436.83 ± 14.25 bc	1476.18 ± 31.47 bc
	MeSA	250.65 ± 5.73 ab	14.94 ± 1.68 b	518.70 ± 21.15 b	1.41 ± 0.18 a	156.51 ± 12.60 b	150.72 ± 8.67 a	469.83 ± 14.25 c	1546.62 ± 107.55 c
	ASA	236.49 ± 9.78 ab	9.18 ± 0.84 a	474.99 ± 13.71 b	1.05 ± 0.15 a	107.43 ± 5.28 a	121.77 ± 8.16 a	392.91 ± 12.27 ab	1364.37 ± 33.51 b
7 days	C	255.18 ± 13.92 a	8.46 ± 1.20 a	587.01 ± 12.27 ab	1.74 ± 0.27 a	156.87 ± 11.58 a	180.93 ± 14.07 a	542.28 ± 15.75 a	1749.42 ± 41.85 a
	SA	301.71 ± 5.73 b	15.69 ± 2.40 a	591.33 ± 20.34 ab	1.92 ± 0.39 a	155.13 ± 13.71 a	169.11 ± 14.46 a	608.91 ± 16.56 ab	1851.06 ± 55.38 a
	MeSA	229.17 ± 7.56 a	12.48 ± 2.52 a	581.13 ± 4.98 a	2.04 ± 0.39 a	162.78 ± 7.56 a	156.21 ± 10.68 a	630.15 ± 34.14 b	1785.81 ± 49.56 a
	ASA	242.55 ± 7.35 a	12.00 ± 1.08 a	648.51 ± 21.87 b	1.98 ± 0.18 a	175.62 ± 10.65 a	175.47 ± 9.42 a	602.97 ± 7.23 ab	1874.10 ± 36.69 a
10 days	C	227.34 ± 4.50 a	9.93 ± 2.64 a	678.24 ± 43.26 a	1.77 ± 0.24 a	171.51 ± 10.35 a	168.39 ± 5.67 a	529.32 ± 12.57 a	1798.98 ± 55.83 a
	SA	235.47 ± 9.99 a	9.51 ± 1.35 a	653.10 ± 27.99 a	2.01 ± 0.21 a	192.21 ± 5.22 ab	161.16 ± 6.33 a	655.95 ± 15.78 b	1919.13 ± 52.95 a
	MeSA	239.61 ± 6.42 a	10.83 ± 1.23 a	726.96 ± 17.25 a	1.86 ± 0.24 a	170.46 ± 4.41 a	168.15 ± 11.37 a	644.13 ± 10.08 b	1973.58 ± 32.58 a
	ASA	230.25 ± 7.20 a	10.77 ± 1.86 a	655.56 ± 23.43 a	2.70 ± 0.48 a	219.03 ± 7.89 b	170.97 ± 14.22 a	678.36 ± 35.25 b	1979.85 ± 79.50 a

Abbreviations: SA—salicylic acid, MeSA—methyl salicylic acid, ASA—acetyl salicylic acid, C—control, h—hours. Data are presented as average value ± standard error (n = 15). Different letters in a table denote significant differences in each phenolic group contents among treatments (C, SA, MeSA, ASA) at each time point, calculated by the Tukey test ($p < 0.05$). [a] Sum of all identified representatives of each individual phenolic group refers to Tables S2–S8. [b] Sum of all identified phenolics in strawberries refers to Tables S2–S8.

The major hydroxycinnamic acid derivative in the strawberry fruits was cinnamoyl hexoside (Table S2), which represented 82–90% of total hydroxycinnamic acid derivatives, followed by less represented chlorogenic acid, caffeic acid derivative and *p*-coumaric acid hexoside. Hydroxycinnamic acid derivatives were clearly most affected when treated with SA with the content increasing by 15–18% in respect to the control. However, the effect lasted during samplings up to 10 days after treatment, with no statistical differences among treatments ($p < 0.05$). A similar trend was also observed in the main representatives of this group on the first sampling date (24 h after treatments).

Catechin and two procyanidin dimers represented the flavanol group in the strawberry fruits (Table S3), whereby catechin was the most present (60–67% of total analysed flavanols). The accumulation of flavanols was significantly ($p < 0.05$) improved by salicylates treatments on most of the sampling dates. The effect was generally greater in the SA and MeSA treatments than for ASA. Compared with the control, SA caused an increase in the flavanol content in the strawberry fruits of up to 27%, MeSA up to 36% and ASA up to 24%. A similar trend was also observed for catechin (Figure 2A) and procyanidin dimer 1, for which a significant ($p < 0.05$) increase was observed in the treatments SA, ASA and MeSA, while other procyanidin dimers were found with a very low content, with no significant differences attributed to the salicylate treatments.

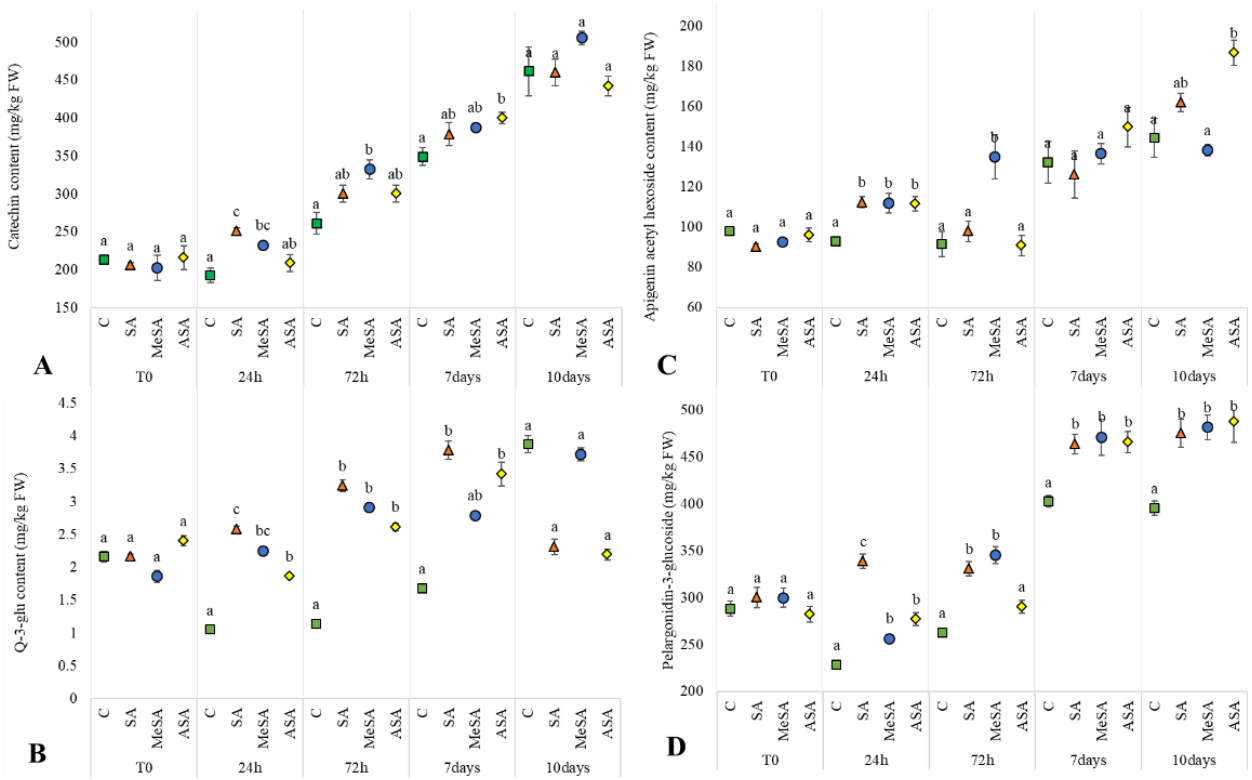

Figure 2. Content of catechin (**A**), quercetin-3-glucoside (**B**), apigenin acetyl hexoside (**C**) and pelargonidin-3-glucoside (**D**) (mg/kg FW) in C-control (■), SA-salicylic acid (▲), MeSA-methyl salicylic acid (●) and ASA-acetyl salicylic acid (♦) treated strawberry fruits at different time points (T0, 24 h, 72 h, 7 days and 10 days after treatments). Different letters (ac) denote a significant difference between treatments (C, SA, MeSA and ASA), obtained with the Tukey test ($p < 0.05$). Data are presented as average value ± standard error ($n = 15$).

Flavonols (Table S4) accounted for a very small share of the phenolic compounds analysed in the strawberries (approximately 0.5–1% TAP). The effects of salicylates on flavonol content were significantly ($p < 0.05$) visible only on the first two sampling dates after treatments (24 h and 72 h). At most sampling dates, an increase in flavonol content was noted

with all salicylate treatments compared to the control. Quercetin-3-glucoside (Figure 2B) was the most abundant flavonol in the strawberries, accounting for 17–34% of all flavonols analysed, followed by quercetin-3-glucuronide (14–30% of total analysed flavonols) and quercetin-3-rutinoside (8–13% of total analysed flavonols). A similar tendency for the content of these three compounds to increase was observed when the strawberries were treated with salicylates. SA had the most marked effect 72 h after treatment, with the quercetin-3-glucoside content on average 2.8-fold higher, quercetin-3-glucuronide content 5.8-fold higher and quercetin-3-rutinoside content 1.6-fold higher than the control.

Among flavones, which represented 8–12% TAP, apigenin acetyl hexoside was the main representative, in the range 75.5–206.4 mg/kg FW (Table S5). The effect of salicylate treatments on apigenin acetyl hexoside was the same as for total flavones (Figure 2C). All salicylates significantly ($p < 0.05$) affected an increase (17%) in total flavones 24 h after treatment. Later, only MeSA increased the level of flavones, at sampling 72 h after treatment (45%) and ASA 10 days after treatment (28%), compared to the control.

Anthocyanins were the major phenolic group in strawberry fruits, with 28–46% TAP with five representatives: cyanidin-3-glucoside, cyanidin-3-*O*-malonylglucoside, pelargonidin-3-glucoside, pelargonidin-3-rutinoside and pelargonidin-3-*O*-malonylglucoside (Table S6). All salicylates contributed to the increase in total anthocyanin content at all sampling dates, with strawberries treated with SA containing up to 51%, strawberries treated with MeSA containing up to 33%, and ASA treated strawberries up to 28% higher anthocyanin content than the control. With a range of 223.6–559.5 mg/kg FW, pelargonidin-3-glucoside (Figure 2D) was the most abundant anthocyanin in the strawberries, which was significantly ($p < 0.05$) affected by salicylate treatments at all sampling dates. No significant effects of salicylate treatment were observed for other anthocyanins, except for SA at 24 h after treatments. Two other phenolic groups (flavanones and ellagic acid derivatives) were identified in the strawberries, which were among the less abundant (Table S7 and S8). In contrast to other phenolic groups, flavanones and ellagic acid derivatives were not affected by salicylate treatments.

4. Discussion

Due to the high perishability and short shelf life of strawberries after harvest, it is necessary to research and use new techniques, including new, alternative chemicals, to maintain quality. Salicylic acid has recently been widely tested on various fruits, since many authors believe that it maintains the quality of various fruits over a longer period of time, regardless of whether they are processed before or after harvest [3,12,25–27]. Some of the most important quality indicators of strawberries, such as fruit colour (L^*, C, h°), fruit weight, fruit firmness and soluble solids content were studied on SA, ASA and MeSA treated fruits. In our case, fruit quality increased, with decreasing hue angle and increasing fruit firmness in almost all treated strawberries, while other indicators remained unchanged. Normally, fruit ripening is accompanied by an increase in weight loss, and in a decrease in fruit firmness and hue angle [28]. In this study, they were mainly delayed by salicylate treatment, especially SA. Similar reports of delayed ripening were obtained by Gimenez et al. [20,21] in sweet cherry cultivars and García-Pastor et al. [25], who reported that SA, ASA and MeSA also led to an increase in total soluble solids and titratable acidity in pomegranate fruits. Babalar et al. [3] showed that treatments with SA improved the overall quality of strawberry fruits, which may be due to its effects on decreasing ethylene production and fungal development. One possible reason could also be inhibition of hydrolytic enzymes such as polygalacturonase, cellulase and xylanase [29].

Among sugars, glucose and fructose were predominant in the strawberry fruits, regardless of treatment, which is in agreement with the strawberry sugar profile described by Mikulic-Petkovsek et al. [30]. The present study showed that all salicylates increased the content of both individual and total sugars in strawberries. Similar results were reported by García-Pastor et al. [25], who showed that the treatments SA, MeSA and ASA also resulted

in higher acids content, while in our study their content was slightly lower or unchanged in the treated fruits.

Ascorbic acid, one of the most important antioxidants that protects organisms against reactive free radicals, is very sensitive to degradation due to its oxidation during fruit processing and storage. In order to maintain the ascorbic acid content in plant foods during storage, various promising pre- and post-harvest tools have been tested, such as thermal treatment [31], high pressure treatments [32], treatments with calcium chloride [33], humic acid [34] and also treatments with salicylic acid [1,25,26,34,35]. We confirmed a positive influence of salicylates on increasing the ascorbic acid content, as reported in most of the mentioned studies, especially in the treatment with SA, which may be related to the effects of SA, a lower activity of ascorbic acid oxidase, which catalyses the oxygenation of ascorbic acid to dehydroascorbic acid, an increase in the activity of ascorbate peroxidase and glutathione reductase or a higher content of reducing sugars [36]. Wisniewska and Chelcowski [37] also found that ascorbic acid content and antioxidant capacity increased in fruits treated with SA, and they concluded that the increased antioxidant capacity of the fruits prevented the destruction of the ascorbic acid.

Phenolic compounds are secondary metabolites that are found in various plant organs and are closely associated with the sensory, nutritional quality of fresh and processed plant foods and their health promoting properties [38]. The present study thus focused on responses to treatments with different salicylic acid derivatives, in terms of the levels of some specific phenolic groups and individual phenolic compounds in the strawberry fruits, using HPLC-MS analysis. After reviewing the different phenolic profiles in strawberries reported by different authors [6,7,25,39], we found that similarities occur, but depending on genetics, environmental conditions, health status and growing technology [4]. In the current study, treatments with salicylates generally resulted in an increase in TAP compared to the control, and better results were obtained with SA and MeSA, which may be due to the role of SA and MeSA in regulating phenolic accumulation by stimulating phenylalanine ammonia lyase (PAL) activity [40,41]. Some recent research has shown that the use of inductors can activate defence mechanisms, such as the phenylpropanoid pathway, which leads to increased synthesis of phenolic compounds, especially flavones, flavonols and flavonoids. Thereby, these metabolites help to protect fruit from pathogens. This was also the case for terpinen-4-ol [42,43]. Increased TAP in treated strawberry fruits may be the reason for the maintenance of ascorbic acid content, since phenolics have a protective effect [35], but this may also be attributed to a delay in the senescence process [26]. Similar effects of SA on total phenolic content have also been shown in table grape [42], cucumber [43], orange [35], pomegranate [25] apricot [40], and peach [44].

To the best of our knowledge, there have been no previous reports in the literature regarding the effect of preharvest treatments with salicylates with a detailed analysis of individual phenolic compounds in strawberry fruits; only a few studies mentioned the beneficial effects of SA on total phenolic, flavonoid and anthocyanin content [13,26,40,45–47]. In total, 34 individual phenolic compounds belonging to seven phenolic groups were identified in the strawberries: hydroxycinnamic acid derivatives, flavanols, flavonols, flavanones, flavones, ellagic acid derivatives and anthocyanins.

Hydroxycinnamic acids (HCAs) are precursors for all other phenolics in the phenylpropanoid pathway and their biosynthesis is regulated by three enzymes, PAL, cinnamate 4-hydroxylase and 4-coumarate-CoA ligase [48]. As mentioned, SA can improve the activity of PAL [40,41]. This was also confirmed in a study of *Salvia miltiorrhiza* cell cultures, while both PAL activity and phenolic acid content were clearly improved with SA treatments [48]. In our study, hydroxycinnamic acid content generally increased in strawberry fruits treated with SA compared to the control, while the effects of MeSA and ASA were not as evident. Gacnik et al. [41] reported that hydroxycinnamic acid increased in apple leaves when they were treated with SA, and especially MeSA, which was further potentiated by the number of MeSA treatments. An increase in HCA content was observed in the main representatives

of this group (chlorogenic acid, caffeic acid derivative and cinnamoyl hexoside) within 24 h after treatment with SA, and also MeSA (caffeic acid derivative, chlorogenic acid).

Total flavanol content was also increased by salicylate treatments and was in general higher for SA treatment than for ASA (after 24 h). A similar trend was observed for catechin and procyanidin dimer 1, which is in agreement with Obinata et al. [49], who found higher contents of catechin and procyanidin in SA treated cultured grape cells than in the control. Ullah et al. [50] reported that SA improved the total flavanol content in poplar by activating the crucial genes for flavanol biosynthesis (MYB134, MYB115, bHLH131 and WD40).

Flavonols and flavones mostly increased with salicylate treatments, while flavanones and ellagic acid derivatives were not affected. Gacnik et al. [41] reported that flavanone contents was not improved with SA and MeSA treatments but, on the other hand, flavonols increased in treated apple leaves. The results suggested that treatments with salicylates may modulate flavonol biosynthesis by activating flavonol synthase activity, which was confirmed in a study on grape berries [51].

Anthocyanins are coloured water-soluble pigments, which are responsible for the red, purple and blue colours of fruits and vegetables [47,49]. We identified five representatives of anthocyanins in strawberries: cyanidin-3-glucoside, cyanidin-3-*O*-malonylglucoside, pelargonidin-3-glucoside, pelargonidin-3-rutinoside and pelargonidin-3-*O*-malonylglucoside. Generally, all salicylates contributed to the increase in total anthocyanins content. Stimulating anthocyanin biosynthesis has also been described in pomegranate fruits [25], ginger [47], cultured grape cells [49], and in callus cultures of *Daucus carota* [52]. The content of pelargonidin-3-glucoside was improved by salicylate treatments, while no other significant effect from the treatments was observed with other anthocyanins. The increased total anthocyanin content in treated fruits resulted in a deeper red colour, which was confirmed by the measured decreasing hue angle. This is a very desirable and important property that the strawberries acquired by treatment with salicylates, since such strawberries are more appreciated by consumers [25].

5. Conclusions

The results of this one-year study suggest that salicylates may improve some highly desirable and important characteristics of strawberries, such as a deeper red colour, delayed ripening by maintaining fruit firmness, and a higher content of sugars, ascorbic acid and phenolics. The mentioned changes could be clear signs that salicylates, in particular salicylic acid, are promising agents for maintaining the quality of strawberries during storage and transport, as well as for improving their nutritional value. The effect of salicylates was generally better, at least in terms of increasing phenolic compound content and some external quality parameters, 24 and 72 h after spraying, while primary metabolite content was better for a longer period after treatment. The conclusion was written based on the results of a one-year study conducted on one strawberry cultivar and with three chemical substances: SA, MeSA and ASA. In the future, the experiment needs to be repeated for another year or two and perhaps extended to different strawberry cultivars. Based on multi-year results, the influence of individual salicylate treatments on the quality parameters of strawberry fruits could be more accurately defined.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae7100400/s1, Table S1: HPLC and HPLC-MS operating conditions for sugars, organic acids and phenolic compounds. Table S2: Content of Identified Flavanols (mg/kg FW) in C, SA, MeSA and ASA Treated Strawberries at Different Time Points (T0, 24 h, 72 h, 7 days and 10 days after treatments with salicylates); Table S3: Content of Identified Flavonols (mg/kg FW) in Salicylic Acid (SA), Methyl Salicylic Acid (MeSA) and Acetyl Salicylic Acid (ASA) Treated Strawberries at Different Time Points (T0, 24 h, 72 h, 7 days and 10 days after treatments with salicylates); Table S4: Content of Identified Flavones (mg/kg FW) in Salicylic Acid (SA), Methyl Salicylic Acid (MeSA) and Acetyl Salicylic Acid (ASA) Treated Strawberries at Different Time Points (T0, 24 h, 72 h, 7 days and 10 days after treatments with salicylates); Table S5: Content of Identified Hydroxycinnamic Acid Derivatives (mg/kg FW) in Salicylic Acid (SA), Methyl Salicylic Acid (MeSA) and Acetyl Salicylic Acid (ASA) Treated Strawberries at Different Time Points (T0, 24 h, 72 h, 7 days and 10 days after treatments with salicylates); Table S6: Content of Identified Anthocyanins (mg/kg FW) in Salicylic Acid (SA), Methyl Salicylic Acid (MeSA) and Acetyl Salicylic Acid (ASA) Treated Strawberries at Different Time Points (T0, 24 h, 72 h, 7 days and 10 days after treatments with salicylates); Table S7: Content of Identified Flavanones (mg/kg FW) in Salicylic Acid (SA), Methyl Salicylic Acid (MeSA) and Acetyl Salicylic Acid (ASA) Treated Strawberries at Different Time Points (T0, 24 h, 72 h, 7 days and 10 days after treatments with salicylates); Table S8: Content of Identified Ellagic Acid Derivatives (mg/kg FW) in Salicylic Acid (SA), Methyl Salicylic Acid (MeSA) and Acetyl Salicylic Acid (ASA) Treated Strawberries at Different Time Points (T0, 24 h, 72 h, 7 days and 10 days after treatments with salicylates).

Author Contributions: S.G. performed all laboratory and statistical analyses, evaluating results and writing this article, R.V. conceived the experiment, M.H. reviewed the manuscript, D.K. prepared plant material, assistance in performing the experiment, M.M.-P. conceived the experiment, assisted with all analyses. All authors reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All data pertaining to this study is being held in computers owned by University of Ljubljana, Ljubljana, Slovenia, under control of the PI team.

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Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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