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Foliar Calcium Effects on Quality and Primary and Secondary Metabolites of White-Fleshed 'Lemonato' Peaches

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Abstract: 'Lemonato' is a Greek peach melting-flesh white-flesh cultivar with high nutritional value highly appreciated by the consumers. This study aimed to evaluate the effect of pre-harvest foliar calcium application on fruit quality, primary metabolite profile, antioxidant activity, total phenolic content, and phenolic profile of the 'Lemonato' peach, clone 'Stamatis'. The experiment was conducted for two years, 2019 and 2020, in two commercial orchards at Kato Lehonia and Agios Vlasios regions, central Greece, where the 'Lemonato' clone 'Stamatis' is traditionally cultivated. The treatments were organic calcium (Ca), calcium–silicate in nanoparticles (Ca–Si), and calcium chloride ($CaCl_2$). Foliar application of the different Ca formulations, commonly used as a horticultural practice, were not effective at improving the fruit quality characteristics in this clone, which is characterized by fruit softening during ripening. The study revealed the sugars and organic acid composition and phenolic profile of the 'Lemonato' peach, clone 'Stamatis'. Peach fruit quality, primary metabolites, and phenolic compounds of the two orchards showed a different response to organic Ca and Ca–Si, indicating that genetic or environmental factors may also be involved. A higher concentration of organic Ca and $CaCl_2$ increased the peach fruit phenolic compounds content and the total antioxidant activity, improving the fruit nutritional quality.

Keywords: calcium chloride; calcium-silicate; firmness; organic acids; phenolic compounds; *Prunus persica*; sugars

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1. Introduction

Peaches (*Prunus persica* L. Batsch) are highly appreciated by consumers for their sensory characteristics, but they are also distinguished for their significant bioactive compound concentrations [1,2]. Many peach cultivars of various peel and flesh color, flavor, and organoleptic quality are cultivated around the world to cover the market and consumer demand. Manganaris et al. [3] highlighted the importance of cultivar breeding programs to focus on the consumer's acceptance and the selection of elite cultivars with enhanced aroma, with appreciable nutritional properties and extended market life. The 'Lemonato' peach is a Greek traditional series of clones with melting-flesh white-flesh peaches that is highly acceptable by consumers due to its distinct flavor, high nutritional value, and high total phenolic content [4,5]. Nevertheless, the main drawback of the 'Lemonato' peach is flesh softening and bruising susceptibility during ripening [5].

In peach, the flavor highly depends on the sugars and organic acid contents, related to sweetness and/or sourness sensation, and phenolic compounds, related to astringency or bitterness [6]. The sweetness intensity depends on the total sugar content and on the specific sugar profile [7], which is the relative content of different sugars present in certain fruit [8]. Peach fruit contains certain soluble sugars and sugar alcohols, mainly sucrose, glucose, fructose, and sorbitol [7]. Mature peach fruit also contains detectable amounts of other sugars such as maltose, isomaltose, raffinose, xylose, trehalose, 1-O-methyl-glucoside and fucose, and the polyols, galactinol, glycerol, myo-inositol, and maltitol [9]. Fruit acidity is also a crucial determinant of peach organoleptic quality with malate, citrate, and quinate being the main organic acids in ripe peach fruit [10].

Phenols act as potent radical scavengers and primary chain-breaking antioxidants [11] and lately, are part of the nutritional quality due to their antioxidant activity, as consumers increasingly demand fruit with more beneficial effects on human health [12]. The most abundant class of phenolic compounds in peach peel and pulp is flavanols (catechin, epicatechin, and procyanidin B1), followed by hydroxycinnamic acids (neochlorogenic and chlorogenic acids) [13]. However, anthocyanidins (cyanidin 3-glucoside and 3-rutinoside) and flavonols (quercetin-3-galactoside, quercetin-3-rutinoside, quercetin-3-glucoside, kaempferol-3-rutinoside, isorhamnetin-3-rutinoside) are also found in peach fruit [11]. The peel contains higher amounts of phenolic compounds compared to the flesh, while several flavonols were detected only in peach peel, with significant differences between the cultivars [14]. Aside from the cultivar, various factors may affect the fruit phenolic content such as climate and cultivation practices [15].

Productivity, fruit quality, and the storage potential of fruit trees are determined by several factors, one of which is tree mineral nutrition. Calcium (Ca) is a major regulatory ion in horticultural crops, having a vital role in fruit ripening through physical and biochemical mechanisms [16]. Ca is an important component of the plant cell wall and binds together pectin strands, helping to maintain fruit firmness postharvest [17–20]. In peach fruit, preharvest Ca sprays were found to positively affect the fruit quality parameters, especially flesh firmness, even after cold storage [21,22].

Calcium is transported via the xylem, and once cell division ceases and subsequent cell expansion begins, very little additional Ca enters the fruit [23]. For this reason, foliar Ca sprays are a typical horticultural practice for various fruit species to improve the fruit cell integrity, disease resistance, quality, or minimize the occurrence of Ca deficiencies [21,24]. However, the response to foliar Ca sprays is variable, and fruit growers often obtain inconsistent results [25].

The effects of the foliar applications of different types and concentrations of Ca formulations (i.e., calcium chloride ($CaCl_2$), Ca–Si in nanoparticles, and organic Ca) on the quality parameters, the antioxidant activity, and the primary and secondary metabolites of 'Lemonato' peach, clone 'Stamatis', in two different orchards were assessed. The combined analyses presented herein provide insights into the metabolic processes linking the peach fruit quality traits with the foliar-based Ca application.

2. Material and Methods

2.1. Plant Material and Experimental Treatments

The experiment took place for two consecutive years, 2019 and 2020, at two commercial orchards of 'Lemonato' peach trees, clone 'Stamatis'. This clone consists of variable genetic material due to the local selection of macroscopically elite cultivated trees to propagate over the last two centuries. The orchards were located at Kato Lehonia and Agios Vlasios regions (Pelion, central Greece) and consisted of 15-year-old trees grafted onto GF-677 rootstock, planted at 5×5 m^2 rectangulars and trained in open vase receiving sufficient cultivation practices. In 2019, the treatments included foliar applications of two Ca formulations, 0.4% v/v calcium–silicate in nanoparticles (Ca–Si, Barrier, Ca: 14.8 w/w), or 0.1% w/v organic Ca (Theocal, organic fertilizer with Ca, Ca: 30% w/w). Control trees remained unsprayed. Five foliar applications were performed, three times with a 20 d interval beginning at petal fall plus two more times during the last 40 d before harvest. In 2020, trees were sprayed with two Ca formulations, calcium chloride 1% *w*/*v* $CaCl_2$ ($CaCl_2$: 77% *w*/*w*) and organic Ca 0.4% *w*/*v* (Theocal), while the control trees remained unsprayed. $CaCl_2$ in 2020 was applied five times in the same way as the Ca formulations in 2019. Organic Ca in 2020 was applied at a higher dose than 2019 and the sprays were repeated six times, four times with a 20 d interval beginning at petal fall plus two more times during the last 40 d before harvest, in both orchards. All Ca formulations used for the experiment were commercial products commonly used in the area. In 2019, fruit was harvested on 26 August 2019 (Kato Lehonia) and 7 September 2019 (Agios Vlasios) and in 2020 on 7 September 2020 (Kato Lehonia) and 14 September 2020 (Agios Vlasios).

2.2. Fruit Quality Characteristics

At harvest, skin and flesh color, flesh firmness (FF), soluble solid content (SSC), and titratable acidity (TA) were evaluated at eight 10-fruit replications per treatment. The color measurements were performed with a Minolta chromameter (Model CR-400, Minolta Ltd., Osaka, Japan) using the CIE a* value. FF was measured after the peel removal at two opposite sides at the equator of each fruit by a digital penetrometer (model 53205, Turoni Srl, Forli, Italy) using an 8.9–mm plunger. Peach juice was extracted from one longitudinal slice of each fruit of the 10-fruit replication. This juice was used to measure SSC with an Atago Refractometer (Model PAL-1, Atago, Tokyo, Japan) and titratable acidity after titration with 0.1 N NaOH to pH 8.2 and proper calculations.

2.3. GC–MS-Based Primary Polar Metabolite Analysis

Peach fruit primary metabolites (300 mg of ground frozen tissue) were extracted with methanol (1.4 mL) plus adonitol (0.1 mL, 0.2 mg mL^{-1}) solution, at 70 °C for 10 min under constant agitation. Thereafter, the metabolite analysis followed a previously reported procedure using a GC PerkinElmer Clarus® 590 equipped with MS Clarus® SQ 8 S (Perkin Elmer, NJ, USA) and a capillary type column (TR-5MS) 30 m × 0.25 mm × 0.25 µm, and the proper calculations as described by Karagiannis et al. [26]. The values were normalized and additionally analyzed by one-way ANOVA followed by the LSD test to detect significant differences ($p < 0.05$). Details regarding the primary metabolic profiling are reported in Supplementary Table S1.

2.4. Total Phenolic Content (TPC) and Total Antioxidant Activity (TAA)

Total phenolic content and TAA analyses were performed at four 10-fruit replications per treatment. Ten slices (pulp and peel) per replication were homogenized and 5 g were extracted with 25 mL methanol, centrifuged at 4000× *g* for 10 min, and the supernatants were analyzed for TPC and TAA using a UV–Vis spectrophotometer (Optizen Pop, Mecacys, Daejeon, Republic of Korea). TPC was measured using the Folin–Ciocalteu reagent as previously described [27]. TAA was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and the ferric ion reducing antioxidant power (FRAP) methods, as previously described [28,29].

2.5. Individual Phenolic Compounds Analysis

Individual phenolic compound analysis was performed in 2020. Three replications for each treatment of frozen ground peach fruit samples were freeze-dried (Freeze-dryer Alpha 1–2 LD plus, Christ, Osterode, Germany; at −24 °C) until a fine powder. One hundred mg of dried sample (peel and pulp) were extracted with 4 mL methanol (80%). The solutions were sonicated for 20 min, shaken for 3 h at 20 °C, left at 4 °C overnight in the dark, filtered through 0.22 µm polytetrafluoroethylene membrane filters into glass vials, and injected directly for polyphenolic analysis. Phenolic compound determination was performed by an ultra-performance liquid chromatography-tandem mass spectrometer (UPLC—MS/MS) on a Waters Acquity system (Milford, MA, USA) using a Waters Acquity HSS T3 column (1.8 µm, 100 × 2.1 mm^2, set at 40 °C) and the separation conditions as previously described by Vrhovsek et al. [30]. Data processing was performed using Mass Lynx Target Lynx Application Manager (Waters).

2.6. Statistical Analysis

Analysis of variance was performed over treatment with the SPSS statistical package (SPSS 29.0, IBM Corp., Armonk, NY, USA). To compare means, the least significant difference (LSD) at $p \leq 0.05$ was used.

Heatmap diagram of the primary polar metabolite levels, correlation analysis, and principal component analysis (PCA) were conducted with ClustVis (biit.cs.ut.ee/clustvis), a web tool for visualizing the clustering of multivariate data.

3. Results and Discussion

3.1. Fruit Quality Characteristics

In 2019, in both regions, peach fruit treated with organic Ca or Ca–Si had a similar peel color a*, flesh color a*, SSC, and acidity with the control fruit (Tables 1 and 2). In 2019, the flesh firmness was similar among the treatments at the Kato Lehonia orchard, while at Agios Vlasios, fruit sprayed with organic Ca had lower flesh firmness than the control fruit (Tables 1 and 2).

Table 1. The effect of calcium formulation application on the fruit quality at Kato Lehonia orchard. Ca–Si: calcium and silicon nanoparticles.

Treatment	Skin a*	Flesh a*	Flesh Firmness (kgF)	SSC (%)	Acidity (%)
			2019		
Control	−13.0 a	−12.2 a	5.32 a	11.6 a	1.16 a
Organic Ca	−12.0 a	−12.0 a	4.70 a	12.1 a	1.16 a
Ca–Si	−12.9 a	−11.8 a	3.92 a	11.8 a	1.11 a
Significance	NS	NS	NS	NS	NS
			2020		
Control	−11.3 b	−10.4 ab	3.80 b	11.8 ab	0.92 a
Organic Ca	−9.32 a	−10.9 b	5.10 a	12.3 a	0.92 a
$CaCl_2$	−9.48 a	−9.80 a	3.30 b	11.4 b	0.85 a
Significance	***	*	***	*	NS

Means followed by different letters within the same column per year show significant differences according to the LSD test. NS, not significant, * $p \leq 0.05$ and *** $p \leq 0.001$.

Table 2. The effect of calcium formulation application on the fruit quality at Agios Vlasios orchard. Ca–Si: calcium and silicon nanoparticles.

Treatment	Skin a*	Flesh a*	Flesh Firmness (kgF)	SSC (%)	Acidity (%)
			2019		
Control	−7.64 a	−9.66 a	3.44 a	12.5 a	0.95 a
Organic calcium	−4.14 a	−8.30 a	2.88 b	12.6 a	0.82 a
Ca–Si	−6.08 a	−8.56 a	3.13 ab	12.8 a	0.92 a
Significance	NS	NS	*	NS	NS
			2020		
Control	−8.49 a	−7.99 a	3.20 b	12.3 b	0.69 b
Organic calcium	−9.51 b	−8.04 a	2.40 b	11.6 c	0.74 ab
$CaCl_2$	−11.5 c	−7.92 a	4.70 a	13.0 a	0.79 a
Significance	***	NS	***	***	**

Means followed by different letters within the same column per year show significant differences according to the LSD test. NS, not significant, * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

In 2020, the foliar application of organic Ca (applied at higher concentration compared to 2019) and $CaCl_2$ differently affected the fruit peel color in the two regions (Tables 1 and 2). At the Kato Lehonia orchard, Ca spraying led to less green skin color, while at Agios Vlasios, Ca application resulted in fruit with a greener skin color, especially following the $CaCl_2$ treatment. Val and Fernández [24] previously reported that $CaCl_2$ had no effect on the L* (lightness), a* (redness), and b* (yellowness) parameters of the peach fruit skin. In 2020, organic Ca or $CaCl_2$ increased the FF in the fruit of Kato Lehonia or Agios Vlasios, respectively. Similarly, in previous studies, $CaCl_2$ application increased FF [22,31]. In another study, the control and Ca-sprayed peaches had similar FF at harvest, but after cold storage, the Ca-sprayed fruit had higher FF values compared to the untreated peaches [21]. At Kato Lehonia, the fruit treated with organic Ca had a higher soluble solid content compared to the fruit treated with $CaCl_2$ (with the control fruit having intermediate values) and there were no differences among the treatments in fruit juice TA. At the Agios Vlasios orchard, the $CaCl_2$ foliar sprays increased the fruit SSC, while organic Ca resulted in lower fruit SSC values compared to the control and $CaCl_2$ treated fruit. $CaCl_2$ increased the fruit juice TA compared to the control. In general, the effect of foliar Ca applications on the fruit quality was not clear. Ali et al. [22] and Val and Fernández [24] mentioned that $CaCl_2$ did not influence SSC, while Wahab et al. [31] observed an increase in SSC with fruit from different peach cultivars. Moreover, the TA level in peach fruit was not affected [21], increased [22], or decreased after $CaCl_2$ foliar applications [31].

3.2. Primary Metabolites

In 2019, the primary metabolites were analyzed using a GC-MS approach to understand the response of peach fruit to Ca applications. It was the first time that the primary metabolites of 'Lemonato' peach have been studied. In peach fruit from both orchards, sucrose was the major sugar followed by fructose and then glucose, while other sugars such as talose, turanose, ribose, arabinose, trehalose, cellobiose, lactose, and xylose were also detected. Sorbitol was the major sugar alcohol in peach fruit, while myo-inositol and arabinitol were also present (Figure 1 and Table S1). Our results are in agreement with Saidani et al. [14], who found that in peach pulp, sucrose was followed by fructose, glucose, and sorbitol as the main sugars and sugar alcohol. It is known that in peach mesocarp, sucrose is the predominant sugar at ripening, followed by glycose and fructose in variable ratios, while sorbitol accounts for less than 10% of the total sugar amount [7]. In the 'Lemonato' peach fruit of both orchards, fructose was found at higher levels compared to glucose, while sorbitol was found at similar levels with glucose (Figure 1 and Table S1). Peach fruit sweetness is positively related to consumer acceptability [32]. Peaches with high eating quality have relatively high fructose content and low glucose and sorbitol contents [7,33].

Acidity strongly affects the sweetness perception of the peach fruit [7]. The comparison of chemical analysis and sensory profiles revealed that sweetness is significantly correlated with the sugar to acid ratio, the total organic acid concentration, and the content of citric and shikimic acids [34]. In 'Lemonato' peach fruit, it was found that malic acid has the most abundant organic acid, followed by quinic acid, and in a lower concentration, citric acid, while other organic acids such as oxalic acid, succinic acid, and erythronic acid were identified in minor quantities (Figure 1 and Table S1). Similarly, a study of the content and profile of organic acids in the ripe fruit of several peach cultivars revealed that malic, citric, and quinic were the major organic acids present [10].

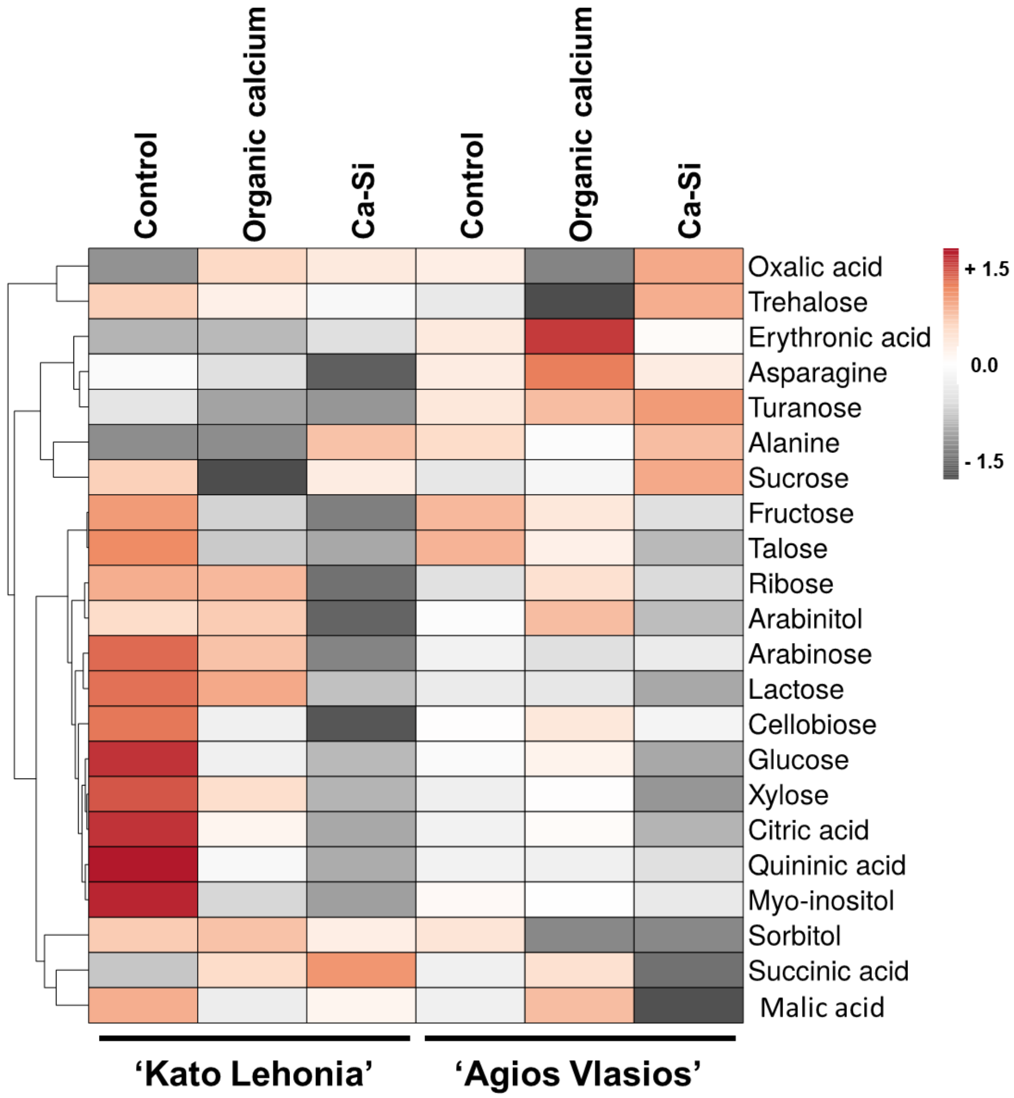

Figure 1. Heatmap diagram of the primary polar metabolite levels in the untreated (control) and in fruit treated with organic calcium and Ca–Si nanoparticles, at the Kato Lehonia and Agios Vlasios orchards in 2019. Each value represents the mean of the relative abundance of adonitol (100 µg mL^{-1}).

At the Kato Lehonia orchard, peach fruit treated with Ca had lower fructose, glucose, talose, ribose (only in Ca–Si), and lactose (only in Ca–Si) levels compared to the control, while sucrose, turanose, arabinose, trehalose, cellobiose, xylose, sorbitol, myo-inositol, and arabinitol were similar among the treatments applied (Figure 1 and Table S1). At Agios Vlasios, peach fruit sprayed with Ca had lower fructose, talose (only in Ca–Si), and sorbitol, while the rest of the sugars and sugar alcohols were similar among treatments (Figure 1 and Table S1). The reduction in the sugar content such as fructose and glucose due to Ca foliar application is evidence that Ca may have delayed fruit ripening, even though this was not clear from the fruit quality characteristics in 2019 (Tables 1 and 2). In peach fruit, sucrose, glucose, and fructose were found to continuously increase during fruit development until harvest, while sorbitol decreased during ripening [9]. Moreover, sugar accumulation in fruit is a complex quantitative trait affected by environmental conditions (i.e., altitude, precipitation etc.), which depends on many interconnected physiological and metabolic processes, and is controlled by multiple genetic and enzymatic responses that interact with the environmental conditions and crop management [7,35].

Regarding the organic acids, at the Kato Lehonia orchard, peach fruit treated with Ca exhibited lower values of citric and quinic acids and higher values of succinic acid compared to the control (Figure 1 and Table S1). At Agios Vlasios, the peach fruit treated with Ca–Si had lower malic and succinic acid, and similar to the rest of the organic acids with the control, while organic Ca had no effect on the organic acid content of peach fruit compared to the control (Figure 1 and Table S1). Organic acid relative content changes in peach fruit as a result of foliar Ca sprays were not associated with fruit ripening. In a previous work with immature fruit, malic and quinic acid concentrations were high, but they decreased during fruit maturation; however, citric acid reached the maximum content at intermediate maturity [36]. Lombardo et al. [9] found that the citric acid levels varied during fruit development and ripening and finally decreased at harvest, while other organic acids such as benzoic, fumaric, quinic, and malic acid levels did not significantly change during peach fruit development and ripening. Zheng et al. [10] found that peach cultivars of low-acid and high-acid content presented a significant reduction or slight changes in the organic acid content, respectively, during the later stages of fruit development.

In 'Lemonato' peach fruit, the amino acids asparagine and alanine were identified using GC-MS analysis (Figure 1 and Table S1). Asparagine is one of the primary forms of organic nitrogen transport and storage in plants [37] and it is the most abundant free amino acid in peach flesh [38]. At Kato Lehonia, peach fruit sprayed with Ca–Si had a lower value of asparagine and a higher value of alanine compared to the control (Figure 1 and Table S1). Amino acids influence the peach flavor and alanine is associated with fruit sweetness [37]. At Agios Vlasios, Ca applications did not affect the asparagine and alanine levels (Figure 1 and Table S1).

A principal component analysis implemented on the detected compounds indicated a clear separation between the two orchards, explained by 22.4% of the variance (PC2) (Figure S1). Moreover, a clear separation was indicated between organic Ca and Ca–Si treatment for both the Kato Lehonia and Agios Vlasios fruits, explained by 51.8% of the variance (PC1) (Figure S1).

A correlation analysis of all primary metabolites with fruit quality characteristics showed that at Kato Lehonia, malic acid had a negative correlation with peach skin color a*, while flesh color a* also had a negative correlation with sorbitol (Figure S2). Ribose, arabinitol, erythronic acid, and alanine affected the titratable acidity of the fruit from Kato Lehonia (Figure S2).

At Agios Vlasios, cellobiose was positively correlated with skin color a* (Figure S3). Flesh firmness and titratable acidity were significantly affected by primary metabolites trehalose and arabinitol (Figure S3). Malic acid and succinic acid indicated a negative correlation with the SSC (Figure S3).

3.3. Total Antioxidant Activity, Total and Individual Phenolic Contents

TAA (DPPH and FRAP assays) and TPC decreased in peaches treated with either Ca form, organic Ca, and Ca–Si, in 2019 at the Kato Lehonia orchard (Table 3). In 2019, at Agios Vlasios, peach fruit treated with organic Ca had lower antioxidant activity with the DPPH assay, but similar to the FRAP assay compared to the control and a similar total phenol content with the control (Table 4). In 2019, at Agios Vlasios, peach fruit treated with Ca–Si had similar TAA (DPPH and FRAP assays) with the control, but higher TPC compared to the control (Table 4). In 2020, at the Kato Lehonia experiment, organic Ca (higher concentration than 2019) increased fruit TAA with the DPPH and FRAP assays, while $CaCl_2$ had no significant impact on TAA compared to the control (Table 3). However, in 2020, at Agios Vlasios, the two Ca formulations led to higher TAA values with both assays compared to the control fruit (Table 4). Similar differences to the antioxidant activities were observed for the fruit TPC between the Ca treated fruit and the control fruit in the two orchards. Apples sprayed with nano-Ca or $CaCl_2$ had increased fruit firmness, titratable acidity, TPC, and TAA compared to the control fruit [39]. Madani et al. [19] reported that $CaCl_2$ could increase the TAA and TPC in papaya fruit. Moreover, Mokrani et al. [11]

found a significant correlation between TPC and TAA in peach, certifying that phenolic compounds contribute to antioxidant capacity. In our study, in 2020, both Ca formulations in the applied concentrations had a significant positive impact on fruit TAA and TPC.

Table 3. The effect of Ca formulation on the total antioxidant activity (DPPH and FRAP assays) and total phenolic content (TPC) at the Kato Lehonia orchard.

Treatment	DPPH (μmol asc. acid/g fw)	FRAP (μmol asc. acid/g fw)	TPC (mg gallic acid/g fw)
		2019	
Control	12.10 a	10.40 a	1.61 a
Organic calcium	9.08 b	7.27 b	1.31 b
Ca–Si	5.54 c	4.99 c	1.06 c
Significance	***	***	***
		2020	
Control	7.37 b	8.17 b	1.31 b
Organic calcium	9.65 a	13.41 a	1.93 a
$CaCl_2$	6.97 b	7.53 b	1.22 b
Significance	***	***	***

Means followed by different letters within the same column show significant differences according to the LSD test. *** $p \leq 0.001$.

Table 4. The effect of the Ca formulation on the total antioxidant activity (DPPH and FRAP assays) and total phenolic content (TPC) at the Agios Vlasios orchard.

Treatment	DPPH (μmol asc. acid/g fw)	FRAP (μmol asc. acid/g fw)	TPC (mg gallic acid/g fw)
		2019	
Control	6.92 a	8.36	0.83 b
Organic calcium	5.88 b	8.04	0.93 b
Ca–Si	7.45 a	8.57	1.07 a
Significance	*	NS	**
		2020	
Control	7.78 b	11.8 b	1.10 b
Organic calcium	9.86 a	12.6 a	1.42 a
$CaCl_2$	9.25 a	12.4 a	1.32 a
Significance	*	*	**

Means followed by different letters within the same column show significant differences according to the LSD test. NS, not significant, * $p \leq 0.05$ and ** $p \leq 0.01$.

In 2020, individual phenolic compounds were identified by UPLC-MS/MS in an attempt to understand the effect of Ca on the secondary metabolism of 'Lemonato' peach fruit. Catechin, epicatechin, procyanidin B1, B2, and B4, neochrorogenic acid, cryptochlorogenic acid, and chlorogenic acid were the major phenolic compounds detected in the fruit (Tables 5 and 6). Procyanidin B1 was the most abundant compound, followed by chlorogenic acid and catechin. Similarly, Mokrani et al. [11] mentioned that flavanols (procyanidin dimers and catechin) were the main phenolic compounds in peach fruit, followed by hydroxycinnamic acids (neochlorogenic and chlorogenic acid), while epicatechin was detected in low concentrations. Flavanols and hydroxycinnamic acids are found in both the peach peel and flesh. In accordance with our study, Ceccarelli et al. [13] observed that procyanidin B1 and chlorogenic acid were the main flavanols and hydroxycinnamic acid, respectively, in peach fruit.

Table 5. The effect of the calcium formulation application on the major individual phenolic compounds at the Kato Lehonia orchard in 2020.

Treatment	Catechin	Epicatechin	Procyanidin B1	Pro-cya-nidin B2 and B4	Neochlorogenic Acid	Cryptochlorogenic Acid	Chlorogenic Acid
	μg/g Dry Weight						
Control	91.5 b	5.2 c	222 c	2.3 b	181 c	17.1 b	252 c
Organic Ca	287 a	23.8 a	2230 a	49.5 a	396 a	22.6 a	609 a
$CaCl_2$	236 a	17.2 b	1550 b	38.0 a	272 b	15.0 b	411 b
Significance	*	**	***	**	**	**	**

Means followed by different letters within the same column show significant differences according to the LSD test. * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

Table 6. The effect of the calcium formulation application on the major individual phenolic compounds at the Agios Vlasios orchard in 2020.

Treatment	Catechin	Epicatechin	Procyanidin B1	Procyanidin B2 and B4	Neochlorogenic Acid	Cryptochlorogenic Acid	Chlorogenic Acid
	μg/g Dry Weight						
Control	246 a	17.3 a	1440 c	29.9 a	286 b	14.9 b	482 b
Organic Ca	312 a	18.5 a	1850 a	43.1 a	356 a	17.1 a	522 b
$CaCl_2$	284 a	17.1 a	1650 b	24.0 a	291 b	15.3 b	607 a
Significance	NS	NS	**	NS	**	*	*

Means followed by different letters within the same column show significant differences according to the LSD test. NS, not significant, * $p \leq 0.05$ and ** $p \leq 0.01$.

In our study, isorhamnetin-3-rutinoside ranged from 5.5 to 16.0 μg/g dry weight. Quercetin-3-rutinoside, quercetin-3-galactoside, quercetin-3-glucoside, kaempferol-3-rutinoside, phlorizin, p-hydroxybenzoic acid, caffeic acid, cyanidin-3-galactoside, and arbutin were also detected in concentrations lower than 10 μg/g dry weight. Quercetin-3-rutinoside, quercetin-3-galactoside, quercetin-3-glucoside, kaempferol-3-rutinoside, and cyanidin-3-galactoside were also identified in different peach cultivars, but phlorizin, p-hydroxybenzoic acid, and caffeic acid were not detected [11].

Ca application significantly increased the phenolic content of peach fruit from both orchards (Tables 5 and 6). At the Kato Lehonia region, organic Ca and $CaCl_2$ enhanced the accumulation of all of the detected phenolic compounds. In general, the use of organic Ca led to the highest values of phenols as the phenolic compounds found in fruit from organic Ca were usually higher than the respective phenolics found in the $CaCl_2$ treated fruit, except for catechin and procyanidins B2 and B4. However, at Agios Vlasios, catechin, epicatechin, and procyanidins B2 and B4 were not affected by the Ca treatments. Moreover, neochlorogenic and cryptochlorogenic acids increased after the application of organic Ca, while the accumulation of chlorogenic acid was enhanced by $CaCl_2$. In a previous study, $CaCl_2$ foliar application increased the phenolic content of olive fruit [40]. In our study, higher values of individual phenolics were usually found in the fruit from Agios Vlasios orchard. Nonetheless, the maximum concentrations of procyanidin B1 (2230 µg/g dry weight) and chlorogenic acid (609 µg/g dry weight) were detected in fruit treated with organic Ca at the Kato Lehonia orchard. In general, Ca application was more effective at increasing the individual phenolics in fruit from the Kato Lehonia orchard.

A principal component analysis performed indicated a clear separation of the investigated treatments (organic calcium and $CaCl_2$ treatment) for both areas of experimentation (Kato Lehonia and Agios Vlasios), and explained 87.8% of the total variance determined (PC2 = 35.4%, PC1 = 52.4%) (Figure S4).

4. Conclusions

The study showed that foliar sprays with organic Ca, Ca–Si in nanoparticles, or $CaCl_2$ were not effective at improving the fruit quality characteristics, especially fruit firmness, of this melting flesh peach cultivar 'Lemonato', clone 'Stamatis'. Peach fruit analysis for the primary metabolites showed that sucrose was the predominant sugar, followed by fructose, glucose, and sorbitol. Malic acid was the most abundant organic acid followed by quinic acid and citric acid. The effect of Ca applications on the peach fruit sugars and organic acids contents was not clear. The total antioxidant activity and total phenolic content increased with the application of high organic Ca concentration and $CaCl_2$ (in one orchard). Procyanidin B1, chlorogenic, and neochlorogenic acids were the main phenolic compounds detected in 'Lemonato' peaches and Ca foliar application (especially the organic Ca) increased the accumulation of these phenolics. This work provides insights into the metabolic shifts and quality traits occurring in peach fruit following various calcium formulation applications in two different orchards.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9030299/s1, Figure S1: Principal component analysis for the primary metabolites and calcium treatment of the two orchards Kato Lehonia and Agios Vlasios; Figure S2: Correlation analysis between primary metabolites and fruit quality characteristics of Kato Lehonia orchard; Figure S3: Correlation analysis between primary metabolites and fruit quality characteristics of Agios Vlasios orchard; Figure S4: Principal component analysis for the individual phenolic compounds and calcium treatment of the two orchards Kato Lehonia and Agios Vlasios; Table S1: Quantitative results of peach fruit primary metabolite analysis.

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