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Foliar calcium effects on quality and primary and secondary metabolites of white-fleshed 'Lemonato' peaches

Persefoni Maletsika [1,*], Vasiliki Liava [1], Eirini Sarrou [2], Vaia Styliani Titeli [3], Elpida Nasiopoulou [3], Stefan Martens [4], Evangelos Karagiannis [3,5], Katerina Grigoriadou [2], Athanassios Molassiotis [3] and George D. Nanos [1]

[1] Laboratory of Pomology, School of Agricultural Sciences, University of Thessaly, Fitoko Str, 38446 Volos, Greece; pmalets@uth.gr
[2] Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organization - DIMITRA, Thermi 57001, Thessaloniki, Greece; katgrigoriadou@elgo.gr
[3] Laboratory of Pomology, Department of Horticulture, Aristotle University of Thessaloniki, Thessaloniki-Thermi 57001, Greece; amolasio@agro.auth.gr
[4] Fondazione Edmund Mach, Centro Ricerca e Innovazione, Department of Food Quality and Nutrition, Via E. Mach, 1, 38010, San Michele all'Adige, TN, Italy; stefan.martens@fmach.it
[5] Department of Agriculture, University of Western Macedonia, 53100 Florina, Greece (Current address); ekaragiannis@uowm.gr
* pmalets@uth.gr; Tel.: +30 2421093175

Abstract: 'Lemonato' is a Greek peach cultivar highly acceptable by the consumers with high nutritional value. This study aimed to evaluate the effect of pre-harvest calcium application on fruit quality, sugars and organic acids 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, in Pelion, central Greece, where 'Lemonato' clone 'Stamatis' is traditionally cultivated. The treatments were organic calcium, calcium-silicate in nanoparticles (Ca-Si) and calcium chloride ($CaCl_2$). Calcium (Ca) foliar applications significantly altered the organoleptic characteristics of the peaches (only in 2020), some sugars and organic acids as well as the antioxidant activity and the total phenolic content of the fruit. Moreover, the accumulation of phenolic compounds was enhanced with increased organic Ca application rate and $CaCl_2$, especially at 'Kato Lehonia' orchard. The maximum increase in phenolic content was observed for procyanidin B1, which was the main phenolic compound of the peach fruit. Chlorogenic acid, neochlorogenic acid, and catechin were also recorded in high concentrations. This study indicates that Ca application influences the quality, specific sugars and organic acids content and remarkably increases the phenolic content of the 'Lemonato' peaches.

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

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1. Introduction

Peach (*Prunus persica* L. Batsch) is among the most widely consumed fruits in the world. Peaches are summer fruits highly appreciated by the consumers for their sensory characteristics, but they are also known to be significant sources of bioactive compounds [1, 2]. Many peach cultivar of various peel and flesh color, flavor and organoleptic quality are cultivated around the world to cover market demands and consumers' preferences. Manganaris et al. [3] highlighted the importance of cultivar breeding programs to focus on 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, highly acceptable by the consumers, owing to its distinct flavor, high nutritional value, and high total

phenolic content [4, 5]. Nevertheless, the main problem of 'Lemonato' peach is flesh softening during ripening and bruising susceptibility of ripe fruit [5].

In peach, taste largely depends on the water-soluble compounds, such as sugars and organic acids, conferring a sweetness and/or sourness sensation, and phenolic compounds, conferring astringency or bitterness [6]. The sweetness intensity depends on the overall sugar amount as well as on the specific sugar profile [7], which is the relative content of different sugars present in certain fruit [8]. Peach fruit accumulates certain types of soluble sugars and sugar alcohols, mainly sucrose, glucose, fructose, and sorbitol [7]. Peach fruit at the mature stage 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]. In addition, fruit acidity is a crucial determinant of peach organoleptic quality and malate, citrate and quinate are the major components of organic acids in ripe peach fruit [10].

Phenols act as potent radical scavengers and primary chain-breaking antioxidants [11] and lately, as significant quality parameters 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 identified in peach fruit [11]. The peel contains higher amounts of phenolic compounds compared with flesh while several flavonols were detected only in peach peel, with significant differences between the cultivars [14]. Besides cultivar, various factors may affect fruit phenolic content, such as climate, geographical origin, and cultivation practices [15].

The productivity, fruit quality and storage potential of fruit trees are influenced by several factors, one of which is tree mineral nutrition. Peach tree mineral nutrition needs are different among cropping systems, growing areas and cultivars [16]. Calcium (Ca) is a major regulatory ion in horticultural crops, having a vital role in fruit ripening through physical and biochemical mechanisms [17]. Ca is an important component of the plant cell wall and binds together pectin strands helping to maintain fruit firmness. It is also involved in maintaining membrane integrity [18]. Thus, Ca treatment could effectively maintain fruit firmness and delay fruit softening and ripening, thus maintaining postharvest fruit quality for longer [18–21]. In peach fruit pre-harvest Ca was found to positively affect fruit quality parameters, especially flesh firmness, even after cold storage [22, 23].

Calcium is transported via the xylem, and once cell division ceases and subsequent cell expansion begins, very little additional Ca enters the fruit tissues [24]. For this reason, foliar Ca sprays for various fruit species is a typical horticultural practice, which can improve cell integrity, disease resistance, fruit quality, or minimize the occurrence of localized Ca deficiencies in the fruit [22, 25]. However, response to foliar Ca sprays is variable and fruit growers often obtain inconsistent results [26].

The aim of the present study was to assess the effect of 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. The combined analyses presented here provide insights into metabolic processes linking peach fruit quality traits with the foliar-based calcium application.

2. Material and Methods

2.1. Plant material and experimental treatments

The experiment was conducted for two consecutive years, 2019 and 2020, at two commercial orchards of 'Lemonato' peach trees, clone 'Stamatis'. This clone consists of varia-

ble genetic material because each farmer selects bud-wood from his own trees with worthy pomological characteristics to produce new plants 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 x 5 spacing and trained in open vase receiving the local horticultural practices. In 2019 trees were sprayed with 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 Ca: 30% w/w), while other trees remained unsprayed (control). Five foliar applications were performed, three times with 20 d interval beginning at petal fall at fruitlet stage 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 other trees remained unsprayed (control). $CaCl_2$ in 2020 was applied the same way as in 2019. Organic Ca sprays were repeated six times, four times with 20 d interval beginning at petal fall plus two more times during the last 40 d before harvest, in both orchards. In 2019, the fruit were harvested on 26/8/2019 ('Kato Lehonia') and 7/9/2019 ('Agios Vlasios') and in 2020 on 07/09/2020 ('Kato Lehonia') and 14/9/2020 ('Agios Vlasios').

2.2. Fruit quality characteristics

At harvest, skin and flesh color, flesh firmness (FF), soluble solids content (SSC), and titratable acidity (TA) were measured at eight replications of 10 fruit per treatment. The color was measured by a Minolta chroma meter (Model CR-400, Minolta Ltd, Osaka, Japan) using CIE a* value. FF was measured after peel removal at two opposite sides of each fruit by a digital penetrometer (model 53205, Turoni Srl, Forli, Italy) equipped with an 8.9–mm plunger. SSC was measured to the peach juice extracted from one longitudinal slice of each fruit of the 10-fruit replication by an Atago Refractometer (Model PAL-1, Atago, Tokyo, Japan). TA was also measured to the extracted peach juice after titration with 0.1 N NaOH to pH 8.2 (expressed in g malic acid per 100 mL juice).

2.3. GC–MS-based primary polar metabolite analysis

Peach primary metabolites (300 mg of grinded 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 minutes under constant agitation. Thereafter, the supernatant was collected via centrifugation and dH_2O (1.5 mL) plus chloroform (0.75 mL) were added. From the polar upper phase 150 μL were dried, and derivatized with methoxyamine hydrochloride (40 μL, 20 mg mL^{-1}, 37 °C, 120 minutes), plus N-methyl-N-(tri- methylsilyl) tri-fluoroacetamide reagent (MSTFA) (70 μL, 37 °C, 30 minutes). One (1 μL) of the primary metabolite extracts were injected at a GC PerkinElmer Clarus® 590 equipped with MS Clarus® SQ 8 S (Perkin Elmer, USA). Capillary type column (TR-5MS) 30 m × 0.25 mm × 0.25 μm was used and the split ratio was set at 70:1. Injector temperature was set to 220 °C, ion source to 230 °C and interface to 250 °C. A temperature program was set as following: hold at 70 °C for 2 minutes, then at 260 °C with a rate of 8 °C per minute and remain for 18 minutes. The carrier gas flow rate was set at one (1) mL per minute. The record of M/Z was in the range of 50–550. Metabolite peaks were identified from internal standards and/or GOLM and NIST11 databases. The relative amounts of the detected metabolites were normalized based on the relative response of the internal standard (adonitol) peak as described by Karagiannis et al. [27]. The values were normalized and additionally analyzed by one-way ANOVA followed by LSD test to detect significant differences ($P < 0.05$). Details regarding the primary metabolic profiling reported in Table 3.

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

TPC and TAA analysis were performed at four replications of 10 fruit per treatment. Ten slices of ten fruit (pulp and peel) per replication were homogenized and 5 g were extracted with 25 mL methanol, centrifuged at 4000g for 10 min, and the supernatants

were analyzed for TPC and TAA. TPC was measured using the Folin-Ciocalteu reagent at 760 nm with a UV–vis spectrophotometer (Optizen Pop, Mecacys, Korea) and expressed as mg of equivalent gallic acid per g of fresh weight based on calibration curve using gallic acid [28]. TAA was evaluated by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity [29] and the Ferric ion Reducing Antioxidant Power (FRAP) [30]. In DPPH and FRAP assays, the absorbance was measured at 517 nm and 593 nm, respectively, using the above-mentioned UV–vis spectrophotometer. DPPH and FRAP results were expressed as μmol of equivalent ascorbic acid per g of fresh weight. Calibration curves were constructed based on the absorbance of ascorbic acid (Asc) standard solutions.

2.5. Individual Phenolic Compounds Analysis

Individual phenolic compounds analysis was performed in 2020. Three replications for each treatment of frozen ground peach 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 the sample (peel and pulp) were subjected to extraction with 4 mL methanol (80%). Then, 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 compounds determination was performed by 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, set at 40 °C), as previously described by Vrhovsek et al. [31]. During the analysis, samples were kept at 6 °C. Water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid were the two mobile phases, the flow was 0.4 mL/min, and the elution was gradient. The gradient profile was 0 min, 5% B; from 0 to 3 min, linear gradient to 20% B; from 3 to 4.3 min, isocratic 20% B; from 4.3 to 9 min, linear gradient to 45% B; from 9 to 11 min, linear gradient to 100% B; from 11 to 13 min, wash at 100% B; from 13.01 to 15 min, back to the initial conditions of 5% B.

A Waters Xevo TQMS (Milford, MA, USA) instrument equipped with an electrospray (ESI) source was used for mass spectrometry detection. Capillary voltage was 3.5 kV in positive mode and −2.5 kV in negative mode; the source was kept at 150 °C; desolvation temperature was 500 °C; cone gas flow, 50 L/h; and desolvation gas flow, 800 L/h. Unit resolution was applied to each quadrupole. Flow injections of each individual metabolite were used to optimize the MRM conditions. For the majority of the metabolites, this was done automatically by the Waters Intellistart software, whereas for some compounds the optimal cone voltages and collision energies were identified during collision-induced dissociation (CID) experiments and manually set. A dwell time of at least 25 ms was applied to each MRM transition. Data processing was performed using the Mass Lynx Target Lynx Application Manager (Waters).

2.6. Statistical analysis

Analysis of variance was conducted over treatment with SPSS statistical package (SPSS Statistics for Windows, Version 29.0, IBM Corporation, Armonk, NY, USA). The differences among treatments were evaluated by using the least significant difference (LSD) for $p \leq 0.05$ level of significance.

3. Results and discussion

3.1. Fruit quality characteristics

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

In 2020, foliar application of organic Ca (that 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 'Kato Lehonia' orchard, Ca spraying led to less green skin color, while at 'Agios Vlasios' the Ca application resulted in fruit with greener skin color, especially following the $CaCl_2$ treatment. However, Val and Fernández [25] reported that $CaCl_2$ had no effect on L* (lightness), a* (redness) and b* (yellowness) parameters of peach fruit skin. Moreover, in 2020, organic Ca or $CaCl_2$ increased FF in the fruit of 'Kato Lehonia' or 'Agios Vlasios', respectively. Similarly, in previous studies, $CaCl_2$ application increased FF [23, 32]. In another study, control and Ca-sprayed peaches had similar FF at harvest but, after cold storage, Ca-sprayed fruit had higher FF values compared to untreated peaches [22]. It was proposed that Ca can maintain fruit texture exerting an important role in cell-to-cell adhesion [20]. Moreover, at 'Kato Lehonia', organic Ca led to higher soluble solid content compared to $CaCl_2$ (with control fruit having intermediate values) and there were no differences among the treatments in the TA of the fruit. At 'Agios Vlasios' orchard, $CaCl_2$ foliar sprays increased SSC, while organic Ca resulted in lower SSC values compared to control and $CaCl_2$ treated fruit. Moreover, $CaCl_2$ increased the TA of the fruit juice compared to control. In general, the effect of Ca foliar fertilization on fruit quality is not clear. For instance, Ali et al. [23] and Val and Fernández [25] mentioned that $CaCl_2$ did not influence total soluble solid concentration, while Wahab et al. [32] observed an increase in peach fruit from different cultivars. Moreover, TA level in peach fruit was not affected [22], increased [23], or decreased after foliar applications of $CaCl_2$ [32].

Table 1. Effect of calcium formulation application on fruit quality at 'Kato Lehonia' orchard. Ca-Si: calcium and silicon nanoparticles.

Treatment	Skin a*	Flesh a*	Flesh firmness (kgF)	SSC (%)	Acidity (%)
			2019		
Control	-13.0a	-12.2a	5.32a	11.6a	1.16a
Organic Ca	-12.0a	-12.0a	4.70a	12.1a	1.16a
Ca-Si	-12.9a	-11.8a	3.92a	11.8a	1.11a
Signif.	NS	NS	NS	NS	NS
			2020		
Control	-11.3b	-10.4ab	3.80b	11.8ab	0.92a
Organic Ca	**-9.3a**	-10.9b	**5.10a**	**12.3a**	0.92a
$CaCl_2$	**-9.5a**	-9.8a	3.30b	11.4b	0.85a
Signif.	***	*	***	*	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. Effect of calcium formulation application on fruit quality at 'Agios Vlasios' orchard. Ca-Si: calcium and silicon nanoparticles.

Treatment	Skin a*	Flesh a*	Flesh firmness (kgF)	SSC (%)	Acidity (%)
			2019		
Control	-7.6a	-9.7a	3.44a	12.5a	0.95a
Organic calcium	-4.1a	-8.3a	**2.88b**	12.6a	0.82a
Ca-Si	-6.1a	-8.6a	3.13ab	12.8a	0.92a
Signif.	NS	NS	*	NS	NS
			2020		
Control	-8.5a	-8.0a	3.20b	12.3b	0.69b
Organic calcium	**-9.5b**	-8.0a	2.40b	**11.6c**	0.74ab

$CaCl_2$	**-11.5c**	-7.9a	**4.70a**	**13.0a**	**0.79a**
Signif.	***	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 *** $p \leq 0.001$.

3.2. Primary metabolites

In 2019, to study the response of peach fruit to calcium applications, primary metabolites were analyzed using GC–MS approach. It was the first time that the primary metabolites of the specific clone of 'Lemonato' peach 'Stamatis' were studied. In peach fruit of both orchards, sucrose was found to be the predominant sugar followed by fructose and then glucose, while other sugars such as talose, turanose, ribose, arabinose, trehalose, cellobiose, lactose and xylose were detected as well. Sorbitol was the major sugar alcohol, while fruit were also found to contain myo-inositol and arabinitol (Table 3). Our results are in accordance 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 major sugar at maturity, followed by glycose and fructose in variable ratios, while sorbitol accounts for less than 10% of the total sugars content [7]. In 'Lemonato' peach fruit of both orchards, fructose was found at higher levels compared to glucose, while sorbitol was found at similar levels with glucose (Table 3). Sweetness is the most important factor affecting consumer acceptability of peaches [33]. Peaches with high eating quality are the ones that have relatively large amounts of fructose and low quantities of glucose and sorbitol [7, 34].

The level of acidity strongly affects the sweetness perception of the peach fruit [7]. The comparison of the chemical analysis and sensory profiles revealed that sweetness is mainly correlated with the ratio between sugars and acids, the overall organic acids concentration, and the amount of citric and shikimic acids [35]. In 'Lemonato' peach fruit, malic acid was the most abundant organic acid followed by quinic acid and, in lower concentration, citric acid, while other organic acids such as oxalic acid, succinic acid and erythronic acid were identified in minor quantities (Table 3). Previously, study of the content and composition of organic acids in ripe fruit of seventy-five peach cultivars revealed that malic, citric, and quinic were the major organic acids [10].

At '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 control, while sucrose, turanose, arabinose, trehalose, cellobiose, xylose, sorbitol, myo-inositol and arabinitol were similar among treatments applied (Table 3). At 'Agios Vlasios', peach fruit sprayed with Ca had lower fructose, talose (only in Ca-Si) and sorbitol, while the rest of sugars and sugar alcohols were similar among treatments (Table 3). The reduction of sugars' content such as fructose and glucose due to Ca foliar application consists of evidence that Ca probably delayed fruit ripening even though this was not clear from 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.), depends on many interconnected physiological and metabolic processes, and controlled by multiple genetic and enzymatic responses that interact with the environment and crop management [7, 36].

Regarding the organic acids, at 'Kato Lehonia' orchard, peach fruit treated with Ca exhibited lower values of citric and quinic acids and higher values of succinic acid compared to control (Table 3). At 'Agios Vlasios', peach fruit treated with Ca-Si had lower malic and succinic acid, and similar the rest of organic acids with control, while organic Ca had no effect on peach fruit organic acids' content compared to control (Table 3). Organic acid relative content changes in peach fruit as a result of foliar Ca sprays were not associated with fruit ripening. In immature fruit, malic and quinic acid concentrations

were high, but they decreased during fruit maturation; however, citric acid reached maximum content at intermediate maturity [37]. Lombardo et al. [9] found that 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 low-acid and high-acid cultivars were characterized by dramatic decrease or slight changes in organic acid content, respectively, during the later stages of fruit development.

Table 3. Primary polar metabolite levels in untreated (control) and in fruit treated with organic calcium and Ca-Si nanoparticles, at 'Kato Lehonia' and 'Agios Vlasios' orchards in 2019. Each value represents the mean of relative abundance of adonitol (100 μg mL^{-1}).

	'Kato Lehonia'			'Agios Vlasios'			Signif.
	Control	Organic calcium	Ca-Si	Control	Organic calcium	Ca-Si	
Soluble sugars and sugar alcohols							
Sucrose	153.6	120.7	148.7	138.9	142.8	157.6	NS
Fructose	40.1a	**29.3c**	**25.1c**	38.6a	35.8ab	**29.9bc**	Signif.
Glucose	31.2a	**24.5b**	**22.2b**	25.1b	26.1b	21.6b	Signif.
Talose	6.40a	**4.40b**	**4.08b**	6.09a	5.43ab	4.25b	Signif.
Turanose	2.15bc	1.38c	1.25c	3.23ab	3.77ab	4.08a	Signif.
Ribose	0.114a	0.113a	**0.0701b**	0.087ab	0.106a	0.086ab	Signif.
Arabinose	0.773	0.722	0.536	0.636	0.603	0.622	NS
Trehalose	0.077	0.067	0.059	0.053	0.021	0.082	NS
Cellobiose	0.574	0.399	0.235	0.428	0.471	0.406	NS
Lactose	0.294a	0.261a	**0.100b**	0.141b	0.135b	0.082b	Signif.
Xylose	0.0189	0.0153	0.0097	0.0121	0.0134	0.0087	NS
Sorbitol	29.9a	30.5a	26.8a	27.9a	**14.7b**	**14.7b**	Signif.
Myo-inositol	2.92	2.135	1.973	2.378	2.34	2.211	NS
Arabinitol	0.0065	0.0067	0.0031	0.0055	0.0069	0.0042	NS
Organic acids							
Malic acid	10.6a	8.17a	9.14a	8.33a	10.5a	**5.37b**	Signif.
Citric acid	5.37a	**4.20b**	**3.24b**	3.89b	4.120b	3.33b	Signif.
Quinic acid	7.46a	**4.73b**	**3.45b**	4.58b	4.54b	4.09b	Signif.
Oxalic acid	0.033	0.062	0.058	0.057	0.032	0.068	NS
Succinic acid	0.142bc	0.164ab	**0.173a**	0.150abc	0.162ab	0.131c	Signif.
Erythronic acid	0.136	0.136	0.141	0.154	0.172	0.150	NS

Means followed by different letters within the same row show significant differences according to the LSD test. NS Not Significant, Signif. significant at $p \leq 0.05$.

3.3. Antioxidant activity, total and individual phenolic content

Antioxidant activity (evaluated by DPPH and FRAP assays) and total phenolic content were both decreased in peaches exposed to either Ca form, organic Ca and Ca-Si in 2019, at 'Kato Lehonia' orchard (Table 4). In 2019, at 'Agios Vlasios', peach fruit treated with organic Ca displayed lower antioxidant activity with the DPPH assay, but similar with the FRAP assay, compared to control and similar total phenol content with control (Table 5). In 2019, at 'Agios Vlasios', peach fruit treated with Ca-Si had similar antioxidant

activity (DPPH and FRAP assays) with control, but higher total phenolic content compared to control (Table 5). In 2020, at 'Kato Lehonia' experiment, organic Ca (higher concentration than 2019) increased the antioxidant activity of the fruit with DPPH and FRAP assays, while $CaCl_2$ had no significant impact compared to control (Table 4). However, in 2020, at Agios Vlasios, the two forms of Ca formulations led to higher values in both assays compared to control fruit (Table 5). Similar differences to antioxidants activities were observed for the total phenolic content of the fruit between the Ca treated fruit and the control fruit in the two orchards. Nano-Ca and $CaCl_2$ spraying in apples increased fruit firmness, titratable acidity, total phenolic content and total antioxidant activity compared to control fruit [38]. Madani et al. [20] reported that $CaCl_2$ can increase total antioxidant activity and phenolic content in papaya fruit. Moreover, Mokrani et al. [11] found a significant correlation between total phenolic content and antioxidant capacity in peach certifying that phenolic compounds contribute to antioxidant capacity. In our study, in 2020 the Ca formulation and the applied concentration (in case of organic calcium) had a significant positive impact on fruit total antioxidant activity and total phenolic content.

Table 4. Effect of Ca formulation on total antioxidant activity (DPPH and FRAP assays) and total phenolic content (TPC) at 'Kato Lehonia' orchard.

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

Means followed by different letters within the same column show significant differences according to the LSD test. NS = Not Significant, *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001.

Table 5. Effect of Ca formulation on total antioxidant activity (DPPH and FRAP assays) and total phenolic content (TPC) at '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
Signif.	*	NS	**
		2020	
Control	7.78b	11.8b	1.1b
Organic calcium	9.86a	12.6a	1.42a
$CaCl_2$	9.25a	12.4a	1.32a
Signif.	*	*	**

Means followed by different letters within the same column show significant differences according to the LSD test. NS = Not Significant, *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001.

In 2020, individual phenolic compounds were identified by UPLC – MS/MS as an attempt to understand the effect of Ca on 'Lemonato' peach fruit secondary metabolism.

Catechin, epicatechin, procyanidin B1, B2, and B4, neochrorogenic acid, cryptochlorogenic acid, and chlorogenic acid were detected in the fruit (Tables 6 and 7). Procyanidin B1 was the most abundant compound, followed by chlorogenic acid and catechin. Similarly, Mokrani et al. [16] 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 both in peach peel and flesh. In accordance with our study, Ceccarelli et al. [13] observed that procyanidin B1 and chlorogenic acid were the main flavanol and hydroxycinnamic acid, respectively, in peach fruit.

Moreover, in our study, isorhamnetin-3-rutinoside ranged from 0.55 to 1.60 mg/100 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 1 mg/100 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 [16].

Table 6. Effect of calcium formulation application on major individual phenolic compounds at 'Kato Lehonia' orchard in 2020.

Treatment	Catechin	Epicatechin	Procyanidin B1	Procyanidin B2 and B4	Neochlorogenic acid	Cryptochlorogenic acid	Chlorogenic acid
	mg/100 g dry weight						
Control	9.15b	0.52c	22.2c	0.23b	18.1c	1.71b	25.2c
Organic Ca	28.7a	2.38a	223a	4.95a	39.6a	2.26a	60.9a
$CaCl_2$	23.6a	1.72b	155b	3.80a	27.2b	1.50b	41.1b
Signif.	*	**	***	**	**	**	**

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

Table 7. Effect of calcium formulation application on major individual phenolic compounds at 'Agios Vlasios' orchard in 2020.

Treatment	Catechin	Epicatechin	Procyanidin B1	Procyanidin B2 and B4	Neochlorogenic acid	Cryptochlorogenic acid	Chlorogenic acid
	mg/100 g dry weight						
Control	24.6a	1.73a	144c	2.99a	28.6b	1.49b	48.2b
Organic Ca	31.2a	1.85a	185a	4.31a	35.6a	1.71a	52.2b
$CaCl_2$	28.4a	1.71a	165b	2.40a	29.1b	1.53b	60.7a
Signif.	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 ** $p \leq 0.01$.

Calcium application significantly increased the phenolic content of peach fruit from both orchards in 2020 (Tables 6 and 7). At 'Kato Lehonia' region, organic Ca and $CaCl_2$ enhanced the accumulation of all detected phenolic compounds. In general, the use of organic Ca led to the highest values of phenols as phenolic compounds found in fruit from organic Ca were usually higher than the respective phenolics found in $CaCl_2$ treated fruit, except for catechin, procyanidin B2 and B4. However, at 'Agios Vlasios', catechin, epicatechin, procyanidin 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, Ca foliar application with $CaCl_2$ increased the phenolic content of olive fruit [39]. 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 (222.80 mg/100 g dry weight) and chlorogenic acid (60.89 mg/100g dry weight) were detected in fruit treated with the organic Ca at 'Kato Lehonia' orchard. In general, the effect of Ca application was more effective to increase individual phenolics in the peaches from 'Kato Lehonia' orchard.

4. Conclusions

The study showed that foliar sprays with organic Ca, Ca-Si in nanoparticles and $CaCl_2$ affected differently the quality of 'Lemonato' peach, clone 'Stamatis'. In general, at 'Agios Vlasios' orchard, Ca foliar application improved peach fruit quality, while at 'Kato Lehonia', organic Ca negatively affected the organoleptic quality. Peach fruit analysis for primary metabolites showed that sucrose was the predominant sugar followed by fructose, glucose and then sorbitol. Malic acid was the most abundant organic acid followed by quinic acid and then citric acid. The effect of Ca applications on peach fruit sugars and organic acids content was not clear. At 'Agios Vlasios' orchard, the antioxidant activity and total phenolic content were increased with the application of high organic Ca concentration and $CaCl_2$, while at 'Kato Lehonia', treatment with $CaCl_2$ had no significant impact. In both experiments, procyanidin B1, chlorogenic, and neochlorogenic acids were the main phenolic compounds detected in 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 application in two different orchard locations.

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