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Research Article

Salicylic acid and nutrient sprays to improve apple fruit quality

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Abstract

Maturity at harvest is one of the most important properties that determine the final quality of the apple fruit. Appearance, texture, flavour and nutritional value are qualities that consumers evaluate empirically to decide whether to eat the fruit again. Within agricultural practices in and out of the orchard, the relationship between plant nutrition and fruit quality is important, and proper nutrient balance is essential to maintain fruit quality. This research work was carried out on 'Golden Delicious' and 'Red Chief' apple trees. A 56 factorial arrangement was used, with 5 concentrations and 6 factors, 25 treatments in a Taguchi L25 structure. Improvements were found in parameters for fruit finish and fruit quality. The factors with the greatest positive effect on quality in order of importance were Salicylic Acid (SA) 1.0 mM, Potassium (K) 58.90 mM, Molybdenum (Mo) 0.516 mM, Calcium (Ca) 70.00 mM and Cobalt (Co) 8.248 mM and in order of importance the following variables: TSS/acidity ratio, total soluble solids TSS, antioxidant capacity (AC), total phenols (TF), and titratable acidity (TA) in 'Golden Delicious' apple. Considering 'Red Chief', the factors Ca 70.00 mM, K 58.79 mM and SA 1.0 mM, with the variables; TA, TSS/acidity ratio, TF, TSS and AC that finally lead to an optimal development of finish and quality in the apple fruit.

Keywords: 'Golden Delicious'; 'Red Chief'; quality; harvest; factors; variables

Introduction

Apples are known for their high antioxidant content, second only to blueberries. Thus, a 100 g consumption of apples can provide free soluble antioxidant equivalent to 180-290 mg of vitamin C (Bouayed *et al.*, 2011).

The antioxidant activity of apple correlates positively with total polyphenolic concentrations, with the concentration of flavonols/procyanidins at the group level and procyanidin B_2 and epicatechin at the individual compound level being the most important antioxidants (Tsao *et al.*, 2005). However, their amount depends mainly on the variety, the part of the fruit studied (Vieira *et al.*, 2011), the environmental conditions, the cultural practices, the stage of maturity at harvest and the storage time (Pissard *et al.*, 2002). Moreover, it is one

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of the most nutritious foods due to its high content of water (>80%), sugars (fructose > glucose > sucrose), organic acids (0.2-0.8%), vitamins (mainly vitamin C 2.3-31.1 mg/100 g dry matter), minerals (ash 0.34%-1.23%) and dietary fibres (2-3%) and pectin (<50% apple fibres), (Kiczorowska and Kikzorowski, 2005). Antioxidants present in apples may decrease the risk of chronic cardiovascular diseases, neurogenerative diseases, stroke, diabetes, obesity and some types of cancer (Bouayed *et al*., 2011).

Maturity at harvest is the most important factor determining the final quality and postharvest life such as appearance, texture, flavour and nutritional value of fruits, however, other factors have important impacts (El-Ramady *et al*., 2018). Bartoshuk and Klee (2013) report that farming practices contribute to flavour, and the flavour quality of many fruits available to consumers has deteriorated. Variability between trees within the same orchard can influence final product quality, as well as, loading levels and fruit distribution can severely affect quality and maturity (Serra *et al*., 2016). The characteristics that determine apple quality can be measured or classified. Consumers initially evaluate the fruit by its external appearance and then by its internal characteristics that give it its eating quality, although the latter can determine whether a customer buys the product again (Musacchi and Serra 2018).

Within farming practices, the relationship between apple tree nutrition and fruit quality is very important, as well as, the right nutrient balance is essential to maintain fruit quality and storage time. The SA and among the nutrients Ca, K, magnesium (Mg), Co and Mo, are considered and correlated with the most notable influence on fruit quality (Casero *et al*., 2004).

The use of SA as a plant hormone, recently is used as a safe compound to maintain postharvest quality, enhance bioactive compounds and delay fruit ripening by exogenous application (Supapvanich and Promyou, 2013). Moreover, it can strengthen fruit tissue structure by maintaining pectin structure (Promyou and Supapvancih, 2016). In wax apple, Supapvanich *et al*. (2017) reported that preharvest applications of SA were effective in delaying loss of firmness, improve antioxidant capacity and increase total phenol content in storage (Khademi and Ershadi, 2013). In peach, SA improved storage capacity and produced a positive effect on bioactive compounds such as total phenol content and antioxidant capacity, providing health benefits (Supapvanich and Promyou, 2013).

Among the nutrients, K is one of the most important components of apple fruit (Brunetto *et al*., 2016). A balance must be achieved with Ca^{2+}, as together they improve quality and storage. Ca is involved in the regulation of enzyme systems and phytohormone activity, increasing tissue resistance to pathogens, as well as postharvest shelf life and nutritional quality (Yfran *et al*., 2017). Timely and balanced supply of Ca sources to fruit crops during the growing season improves shelf life and nutritional quality (Aghdam *et al*., 2012). K is important in solute transport, partitioning of assimilates, and synthesis of polyphenols responsible for colour and aroma. In addition, it positively affects size, firmness, TSS, juiciness and is very important for fruit storage (Brunetto *et al*., 2016). Conversely, its deficiency reduces fruit acidity, causes poor colouring, small fruit and low organic acids content (Musacchi and Serra, 2018).

Ca applied by spraying is the most widely used method to increase its content in fruit, however, its effectiveness is irregular and depends on the number of applications, type of salt and period in which it is applied. In apple, Cepeda *et al*. (2014) report that spraying 4% calcium chloride increased the Ca content, which favours the significant and consistent delay of firmness losses and evolution of the sugar/acidity ratio, as well as the reduction of weight loss.

When it comes to deficiencies, there are varieties such as 'Golden Delicious' that are very susceptible to Mg deficiency, which in turn causes a decrease in productivity and fruit quality. Mg helps to maintain the integrity of the cell wall and the plasma membrane. It is a bivalent cation as Ca^{2+} and it is possible to bound between pectic substances within the cell wall or bound between the polar heads in the plasma membrane (Farag and Neven, 2012). With preharvest applications on apple trees, Farag and Neven (2012) demonstrated that $MgCl_2$ combined with $CaCl_2$ resulted in apples with higher firmness, higher total soluble solids, higher acidity and vitamin C content.

Mo, as an essential micronutrient, can act as an electron carrier in an enzyme system that triggers an oxidation-reduction reaction, which is vital for plant development and reproduction, and is also present in the enzyme nitrate reductase responsible for nitrate reduction in plants (Singh *et al*., 2015). Co^{2+} ion is an inhibitor of the ethylene biosynthesis pathway (Lau and Yang, 1976), through the conversion of methionine to ethylene in apple tissue (Oi-Lim and Shang, 1976), blocking the conversion of 1-amino-cyclopropane-1-carboxylic acid (ACC) which is oxidised and cleaved to ethylene realising CO_2 (Jordan and Casaretto, 2006).

Work with treatments of aminoethoxyvinylglycine and CoCl2 was shown to be accompanied by changes in the activity of a polygalacturonase inhibitor protein (PGIP) during storage of apple fruits. This inhibitor has been previously isolated from apple fruit tissues. PGIP was also shown to inhibit the activity of an enzyme produced by certain non-pathogenic fungi. Therefore, it may be linked to the resistance of apple fruit to these fungi (Bulantseva *et al*., 2001).

Previous work on the application and dosage of SA and nutrients in preharvest sprays in apple has been carried out in the past, the information on their combined application is limited, however these applications are known to be safe and reliable alternative. Therefore, the aim of this study was to evaluate the effects of preharvest sprays of SA and nutrients combined to improve the quality and bioactive compounds of apple cvs. 'Golden Delicious' and 'Red Chief'.

Materials and Methods

Experimental area and treatments

The research work was carried out in the orchard and cold storage plant "La Campana" in the 2019 cycle, with apple trees of the varieties 'Golden Delicious' and 'Red Chief' in full production and population density with 625 trees ha^{-1} located in the Mennonite field 22 in the municipality of Cuauhtémoc, Chihuahua, Mexico, with an average altitude of 2048 meters above sea level, North latitude 28° 26' 17.5" and West longitude 106° 53' 40.3". In the soil laboratory of the faculty of agrotechnological Sciences of the Universidad Autónoma de Chihuahua, the analyses of fruit finish and quality were carried out.

A 56 factorial arrangement was used, with 5 concentrations and 6 factors (Table 1). The experiment was limited to 25 treatments in the Taguchi L25 structure (Table 2) with 3 replications.

Table 1. Factors and levels of application of the Taguchi L25 structure

Levels	Factors Mm					
	K	Ca	Co	Mo	SA	Mg
0	0.000	0.000	0.000	0.000	0.000	0.000
1	5.000	7.000	0.015	0.030	0.100	1.500
5	25.000	35.000	0.075	0.150	0.500	7.500
10	50.000	70.000	0.150	0.300	1.000	15.000
20	100.000	140.000	0.300	0.600	2.000	30.000
Simple mean	50.000	70.000	0.150	0.300	1.000	15.000
Mother solution mM	10.000	50.000	0.500	0.500	0.500	10.000

Sources: Spinning-K[MR] (K, 50.0%); Fender CaB[MR] (Ca, 10.0%); $CoCl_2$ (Co, 24.8%); Prosimol[MR] (Mo, 39.0%); salicylic acid (SA, 99.7%) and $MgSO_4{\cdot}7H_2O$ (Mg, 16.3%).

Table 2. Treatments formed in Taguchi L25 structure, mL of mother solution application, for apple sprays

Treatment	Spinning-K	Fender CaB	$CoCl_2$	Prosimol	SA	$MgSO_4$
1	0.000	0.000	0.000	0.000	0.000	0.000
2	0.000	0.119	0.026	0.051	0.170	0.128
3	0.000	0.595	0.128	0.255	0.850	0.638
4	0.000	1.190	0.255	0.510	1.700	1.275
5	0.000	2.380	0.510	1.020	3.400	2.550
6	0.425	0.000	0.026	0.255	1.700	2.550
7	0.425	0.119	0.128	0.510	3.400	0.000
8	0.425	0.595	0.255	1.020	0.000	0.128
9	0.425	1.119	0.510	0.000	0.170	0.638
10	0.425	2.380	0.026	0.051	0.850	1.275
11	2.125	0.000	0.128	1.020	0.170	1.275
12	2.125	0.119	0.255	0.000	0.850	2.550
13	2.125	0.595	0.510	0.051	1.700	0.000
14	2.125	1.190	0.000	0.255	3.400	0.128
15	2.125	2.380	0.026	0.510	0.000	0.638
16	4.250	0.000	0.255	0.051	3.400	0.638
17	4.250	0.119	0.510	0.255	0.000	1.275
18	4.250	0.595	0.000	0.510	0.170	2.550
19	4.250	1.190	0.026	1.020	0.850	0.000
20	4.250	2.380	0.128	0.000	1.700	0.128
21	8.500	0.000	0.510	0.510	0.850	0.128
22	8.500	0.119	0.000	1.020	1.700	0.638
23	8.500	0.595	0.026	0.000	3.400	1.275
24	8.500	1.190	0.128	0.051	0.000	2.550
25	8.500	2.380	0.255	0.255	0.170	0.000

Indistinctly to each treatment, 2.0 mL of Disapen AC[MR] was added and each treatment was volumetrically diluted with water to 850 mL of solution.

Six separate sprays were made on 17 and 26 July and 2, 9, 17 and 23 August. For each treatment, 3 replications were carried out, then 75 branches of the 'Golden Delicious' variety and 75 variety of the 'Red Chief' variety were selected. Manual sprinklers were used, applying 141.7 mL of solution for each date for each replicate and treatment.

Harvest

Harvesting was carried out on 28 August. In the field, special care was taken to obtain commercial quality fruit, without any physical damage or visible diseases. For each variety, treatment and repetition, 6 fruits were selected and placed in plastic bags and transferred to the soil laboratory, where they were kept at room temperature to simulate the shelf life. At 9 days after harvest, the variables weight, diameter, colour, firmness, juice density and juice percentage were determined for fruit finish and the variables total soluble solids (TSS), titratable acidity (TA), TSS/acidity ratio, total phenols (TF) and antioxidant capacity (AC) for fruit quality.

Fruit finish

The scale developed by Soto *et al*. (2001) was used to obtain colour in percentage (%). Two colour measurements per fruit, being two intermediate sides in terms of colour, considering six categories for 'Golden Delicious': 1) green; 2) rough green rough lenticels; 3) waxy green; 4) transition to yellow colour; 5) whitish yellow (yellowish); and 6) strongly yellow with a tendency towards orange; and six categories for 'Red Chief': 1) green; 2) beginning of red streak formation; 3) uniform dull red streaks; 4) evident dark red streaks; 5) less uniform streaks, dark red beginning; and 6) full dark red. The colour scale was expressed as a percentage, to make the scale more objective.

Weight was determined using an Ohaus scout pro model digital scale, with a capacity of 0.0-600.00 g and expressed in g.

For the diameter, it was obtained from the average of the polar and equatorial diameters, with a Steren vernier calibrator with a range of 0-15.0 cm, expressed in mm.

Fruit firmness was determined with a Wilson FTB 327 hand-held penetrometer with a capacity of 0 to 29 lb in^{-2}, with an 11 mm plunger. Two readings were taken on the sides where colour was measured, the peel was removed for measurement and the two readings were averaged.

For the determination of juice density or juiciness and juice percentage, two segments were extracted from each fruit (one for each pressure test), weighed and taken to the juice extractor. The extract obtained was placed in a graduated cylinder, left to stand until phase separation was observed and the volume of juice and bagasse was quantified. The juice density or juiciness g ml^{-1} and the percentage of juice were obtained.

Fruit quality

For the determination of TSS in °Brix, a Red Rooster model 90681 refractometer was used, scale from 0.0 to 32.0 °Brix, of the extract obtained for the determination of juice density, a few drops were deposited on the prism of the refractometer previously calibrated with distilled water.

The titratable acidity in % of malic acid was obtained using 10 mL of the same juice used to obtain the juice density, 6 drops of 1% phenophthalein were added, and a titration was made with 0.1 N sodium hydroxide, until a brick pink colour was obtained. The volume used was converted to its malic acid equivalent.

The TSS/titratable acidity ratio was determined on the basis of TSS and malic acid.

Total phenols were determined according to the technique of Singleton and Rossi (1965), with slight modifications, using gallic acid as a standard. A quantity of 2 g of apple pulp was ground and extracted with 20 ml of 80% methanol. 750 μl of 2% sodium carbonate, 250 μl of 50% Folin-Ciocalteau, 1375 μl of distilled water and 250 μl of the pulp extract was placed in a test tube. Vortexed and left to react for 60 min in the dark at room temperature. The absorbance was measured at 725 nm in a DR 5000 Hach visible spectrophotometer. Results were expressed as g gallic acid per g fresh weight (μg GA g^{-1} p.f.). A calibration curve was plotted. Linearity was determined between 0.5 and 2.0 mg ml^{-1}, using a high purity reagent grade gallic acid standard, the calibration was measured in triplicate, the value of the equation was 6.2228x - 0.0107, with an r^2 of 0.9804.

For the analysis of antioxidant capacity according to the methodology of Brand-Williams *et al*. (1995), with slight modifications. 2.8 ml of freshly prepared 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (3.94 mg DPPH in 100 ml 80% methanol) was added to a test tube, 0.2 ml supernatant of the homogenate used for the determination of total phenols was added, vortexed and allowed to react for 60 min in the dark at room temperature. The absorbance was measured at 517 nm, using a DR 5000 Hach visible spectrophotometer. As a blank, the extract was replaced with 80% methanol and a capacity curve was plotted. Linearity was determined between 0 and 600 M using high purity reagent grade Trolox as standard, the calibration was measured in triplicate. -The equation had a value of 0.0008x + 0.6984 with an r^2 of 0.9855. The analyses were measured in triplicate. Results were expressed as mg Trolox g^{-1} fresh weight.

Statistical analysis

Given the Taguchi L25 factorial structure for the generation of the treatments, the statistical analysis was performed by linear and full quadratic response surface, adjusting the surface to determine the levels of the factors for optimal response. A response surface was estimated by least squares regression using the SAS statistical package (SAS Institute Inc., SAS/STAT Software: Usage and Reference, Version 6, First Edition, Cary, NC: SAS Institute Inc., 1989). The analysis for each response variable included three stages: 1) analysis of the regression and the contribution of each factor to the regression fit; 2) canonical analysis of the response surface to determine the shape of the curve for those factors that had significant linear, quadratic and interaction responses; and 3) the predicted values depending on whether the minimum or maximum response was selected according to the original range of the data.

The behaviour of all response variables was summarised in a table specifying the factors and the simple average for each of them. The resulting eigenvalues expressed as percentages of the mean are taken as positive or negative, as appropriate. The contribution of the eigenvectors was expressed with rounded signs so that 0.3750 ≤ ++ ≤ 0.6249, 0.6250 ≤ +++ ≤ 0.8749, ++++ >0.8750. The same procedure was applied to the negative eigenvalues. In this way, the factors were weighted to determine which have more influence on each variable.

Results and Discussion

Fruit finish

Table 3 shows the selection of factors and variables for the finishing of apple fruit cv 'Golden Delicious'. In order of importance, the factors with the greatest effect were Ca, Mo, K and SA, to positively influence the variables of weight, colour, diameter, firmness and juice percentage. Table 4 shows the results for the cv 'Red Chief', where the selected factors were Ca, K, SA and Co and the variables with positive effect by the factors in order of importance were colour, juice percentage, weight, diameter, juice density and firmness. These results are from the statistical analysis for fruit finish, Tables 1, 2, 3 and 4 of the supplementary files.

Table 3. Selection of factors and variables for fruit finishing of apple cv. 'Golden Delicious'

Factors / simple mean [mM]								
Eigenvalues	K	Ca	Co	Mo	SA	Mg	Eigenvectors	
	50.00	70.00	0.150	0.300	1.000	15.00	Total	Prop. + / -
Subtotal	19	25	13	22	16	8	103	
Select variables	3 / 6	4 / 6	3 / 6	4 / 6	3 / 6	2 / 6	Variables 5 / 6	
Prop. + / -	10 / 9	21 / 4	8 / 5	17 / 5	13 / 3	8 / 0		77/26
mM []	50.00	70.00		0.300	1.000		Selection ≥ 26 (19)	

Selected factors: Ca [70.00] > Mo [0.300] > K [50.00] > SA [1.000]. Selected variables: Weight (174.23 g) > colour (51.81%) > diameter (71.8 mm) > firmness (15.35 lb in^2) > juice percentage (94.12)

Table 4. Selection of factors and variables for fruit finishing of apple cv. 'Red Chief'

Factors / simple mean [mM]								
Eigenvalues	K	Ca	Co	Mo	SA	Mg	Eigenvectors	
	50.00	70.00	0.150	0.300	1.000	15.00	Total	Prop. + / -
Subtotal	22	25	20	10	21	2	100	
Select variables	4 / 6	5 / 6	4 / 6	2 / 6	4 / 6	0 / 6	Variables 6 / 6	
Prop. + / -	15 / 7	25 / 0	14 / 6	10 / 0	21 / 0	2 / 0		87 / 13
mM []	50.00	92.11	0.148		1.000		Selection ≥ 17 (13)	

Selected factors: Ca [92.11] > K [50.00] > SA [1.000] > Co [0.148]. Selected variables: Colour (65.75%) > juice percentage (95.00) > weight (171.38 g) > diameter (76.0 mm) > juice density (1.36 g ml^{-1}) > firmness (15.98 lb in^2)

Previous studies have shown that Ca sprays on apple did not affect yield parameters, cv 'Golden Delicious' (Torres *et al*., 2017), nor fruit weight in cv 'Jonagold', however, fruits sprayed with Ca contained more of this nutrient and were firmer than the control at harvest (Wójcik and Mieczysław, 2013). Our results showed that applying a concentration of Ca 70.00 mM, Mo 0.300 Mm, K 50.00 Mm and SA 1.000 mM, fruits of 174.23 g can be obtained in cv 'Goden Delicious' and a firmness of 15.35 lb in^2, while with the concentration of Ca 92.11 mM, K 50.00 mM, SA 1.000 mM, and Co 0.148 mM in cv 'Red Chief', fruits remained similar at 171.38 g, with higher firmness 15.98 lb in^2, and linked firmness with high TA, presented an inhibition of the apple ripening process as a result of the sprays. Similar results were obtained as an increase in fruit Ca content was observed more successfully with foliar Ca sprays than with soil applications (Torres *et al.*, 2017) and was positively correlated with higher firmness at harvest (Casero *et al.*, 2013).

Ca content decreases fruit ripening and decay rate, as it may be slowing the breakdown of soluble pectins in the cell wall because they strengthen the ionic bonds between the cell wall molecules (Hussain *et al.*, 2012). Ca also improves fruit firmness by cross-linking pectic polysaccharide chains due to its divalent ionic character, as well as Ca binding to cell wall components. It can also reduce the accessibility of cell wall degrading enzymes to their substrates (Vicente *et al.*, 2009).

On the other hand, Supapvanich *et al.* (2017), with applications of SA 0.5 and 1.0 mM before harvest in apple, were effective in delaying the loss of fruit firmness and there were no significant differences between the two concentrations. Likewise, it had no effect on weight loss and colour changes during storage for 9 days, results that agree with our data obtained with the 1.0 mM concentration for both varieties since the qualities are preserved. Similar works in peach (Muhammad *et al.*, 2012), show that at a concentration of 2.0 mM showed significantly less weight loss, higher pulp content and firmness as well as greater skin brightness.

SA affects cell expansion helping to maintain firmness of harvested kiwifruit (Zhang *et al.*, 2003). Thus, exogenous application of SA can delay membrane dysfunction and increase of soluble pectin in rambutan (Supapvanich, 2015), and also inhibit certain cell wall hydrolases during banana fruit maturation (Srivastava and Dwivedi, 2000).

Colour is recognised as an important attribute of apples (Moneruzzaman *et al.*, 2015), In our results the colour of the fruit cv 'Golden Delicious' was maintained at 51.81% while in cv 'Red Chief' the percentage was 65.75% both at a concentration of 1.0 mM of SA, so we are below the parameters according to NMX-FF-061-2003, since the minimum colour percentages for red varieties in Mexico is 66 to 75% and in yellow and green varieties minimum 75%, coupled with the above environmental conditions and orchard management such as irrigation, pruning and fertilization strongly impact on fruit colour (Bouzayen *et al.*, 2010). In studies by Supapvanich *et al.* (2017) on apples treated with 0.5 and 1.0 mM of SA, during 9 days of storage all colour attributes remained constant. Shafiee *et al.* (2010) with pre-harvest application of SA on strawberry did not change the hue angle of strawberry fruit. Similar results were obtained on rambutan fruit, colour remained constant with SA applications (Supapvancih, 2015). Other studies have shown, that skin colour was not influenced with Ca sprays, but the colouring depended on the spray measures and combination with other nutrients (Wójcik and Mieczysław, 2013).

In mandarin Yfran *et al.* (2017) obtained higher yields by foliar Ca supply with added K, and higher K concentration in the fruit rind than in the control treatment. In apples, improvement of K deficiencies can increase the colour of red fruits (Vicente *et al.*, 2009), but may not be evident when the K level of the tree is adequate.

It is widely known that ethylene is essential for proper fruit ripening and the development of attributes such as colour, however, overripening reduces its shelf life (Jacob *et al.*, 2012). Furthermore, ethylene controls the rate of ripening and most fruit ripening events (Bouzayen *et al.*, 2010), with increased respiration accompanied by increased ethylene causing irreversible changes in firmness (Omboki *et al.*, 2015). The Co^{2+} ion is an inhibitor of the ethylene biosynthesis pathway (Lau and Yang, 1976), so its inclusion in the nutrient solution due to its participation in the oxide-reduction processes, specifically Mo and Co, have been used in raspberry plants to delay ripening and senescence (Navarro, 2003). Our results indicate that colour and firmness attributes were maintained for the 'Golden Delicious' variety with 0.300 mM Mo and for the 'Red Chief' variety with 0.148 mM of Co. Gad and Kandil, (2010) found that the addition of Co had a favourable effect on tomato and cucumber yield (Gad *et al.*, 2018), and chemical components such as TSS.

Fruit quality

Table 5 shows the selection of factors and variables for fruit quality cv ‘Golden Delicious’. The factors with the greatest effect in order of importance were SA 1.0 mM, K 58.90 mM, Mo 0.516 mM, Ca 70.00 mM and Co 8.248 mM, to positively favour and in order of importance the following variables: TSS/acidity ratio, TSS, AC, TF, and AT. Similarly, Table 6 shows the results for the cv ‘Red Chief’, where the selected factors were Ca 70.00 mM, K 58.79 mM and SA 1.0 mM, with the variables; titratable acidity, TSS/acidity ratio, TF, TSS and AC. These results are from the statistical analysis for fruit quality tables 5, 6, 7 and 8 of supplementary files.

Table 5. Selection of factors and variables for fruit quality of apple cv. 'Golden Delicious'

Factors / simple average [mM]								
Eigenvalues	K	Ca	Co	Mo	SA	Mg	Eigenvectors	
	50.00	70.00	0.150	0.300	1.000	15.00	Total	Prop. + / -
Subtotal	22	21	20	22	24	0	109	
Select variables	3 / 5	2 / 5	3 / 5	3 / 5	4 / 5	0 / 5	Variables 5 / 5	
Prop. + / -	20 / 2	19 / 2	18 / 2	12 / 10	18 / 6	0 / 0		87 / 22
mM	58.90	70.00	0.248	0.516	1.000		Selection ≥ 17 (13)	

Selected factors: SA [1.000] > K [58.90] = Mo [0.516] > Ca [70.00] > Co [0.248]. Selected variables: TSS/acidity ratio (22.99) > TSS (12.3 °Brix) > antioxidant capacity (4.438 mg trolox g^{-1} p.f.) > total phenols (676.290 µg gal acid g^{-1} p.f.) > titratable acidity (0.5226% malic acid

Table 6. Selection of factors and variables for fruit quality of apple cv. 'Red Chief'

Factors / simple average [mM]								
Eigenvalues	K	Ca	Co	Mo	SA	Mg	Eigenvectors	
	50.00	70.00	0.150	0.300	1.000	15.00	Total	Prop. + / -
Subtotal	20	22	12	10	13	2	79	
Select variables	4 / 5	5 / 5	3 / 5	3 / 5	3 / 5	0 / 5	Variables 5 / 5	
Prop. + / -	17 / 3	20 / 2	10 / 2	7 / 3	11 / 2	2 / 0		67 / 12
mM	58.79	70.00			1.000		Selection ≥ 13 (10)	

Selected factors: Ca [70.00] > K [58.79] > SA [1.000]. Selected variables: Titratable acidity (0.4228% malic acid) > TSS/acidity ratio (26.12) > total phenols (816.613 µg gallic acid) > TSS (12.4 °Brix) > antioxidant capacity (4.633 mg trolox g^{-1} p.f.)

Our results show a concentration of 1.00 mM of SA for both varieties, in order to maintain the parameters of SA concentrations and being able to conserve the variables of TA, TSS, TF, AC that contribute to maintaining the quality of the apple fruit. According to the Mexican norm NMX-F-045-1982, it establishes that the TA content in apples must be greater than 0.30 and less than 0.60 g malic acid/100 g, which agrees with our results, 0.5226 g malic acid/100 g for the ‘Red Chief’ variety and 0.4228 g malic acid/100 g for the ‘Golden Delicious’ variety. This is mentioned by Shafiee *et al*. (2010), when the TSS and TA contents of the fruit remained constant with applications of 1.00 mM of SA before harvest. The TF content of control fruit was lower than that of SA-treated fruit and remained constant during storage, while that of both SA treated fruit was improved and increased markedly during 9-day storage. It is widely known that exogenous application of SA, induces defence mechanisms and stimulates bioactive compounds including antioxidants, in harvested fruits and vegetables (Supapvanich and Promyou, 2013).

The amount of both AC and DPPH free radical scavenging activity were enhanced by preharvest SA applications. Sarikhani *et al*. (2010) reported that phenylalanine ammonia lyase was stimulated by exogenous application of SA, which led to the improvement of TF. These results are in agreement with the results of Wei *et al*. (2011), Razavi *et al*. (2014), and Promyou and Supapvanich (2016). The activities of fruit antioxidant enzymes catalase activity (CAT), peroxidase activity (POD) and superoxide dismutase (SOD) that benefit human health were improved and lower preharvest concentrations of 0.5 mM of SA were more effective than higher concentrations of 1.0 mM (Supapvanich, 2017). Similarly, preharvest treatments of 0.5 Mm SA induced CAT, POD and SOD activities in cherry fruits during storage. Dokhanieh *et al.* (2013), in cherry, 1.0- and 2.0-Mm SA treatments increased TF during storage, being higher than 2.0 mM. Antioxidant capacity was significantly improved, but higher at the 1.0 mM concentration than at 2.0 Mm.

Bal (2016) concluded that SA plays a very effective role in controlling weight loss in nectarine and other compositional changes such as AT, TSS, TF and AC of nectarine during cold storage at 1.0 mM concentration, ripening was delayed and with minimal quality loss compared to control samples.

At the postharvest stage, SA can activate systemic acquired resistance (SAR) and localized acquired resistance (LAR) inducing H_2O_2 accumulation for stress defence (Asghari and Morteza, 2010). In mandarin with 2.0 mM SA application, Zhu *et al.* (2016), showed that 6 days after treatment, fruit firmness was significantly higher than the control, which indicates high cell wall integrity. In addition, several studies have concluded that SA can repress polygalacturonase and pectin methyl esterase activities to maintain fruit firmness (Asghari and Morteza, 2010).

Promyuo (2016) in papaya with immersions of 1.0- and 2.0-Mm enhanced fruit firmness at both concentrations in comparison to the control, with the highest firmness at 2.0 mM at the end of storage. Moreover, SA treatments delayed degradation of pectinic substances in papaya, was associated with maintenance of cell membrane properties and pectin structures. Similar results were observed in melon (Supapvanich and Tucker, 2011) and jujube fruit (Promyou *et al.*, 2012). Results in papaya showed that SA treatment improved AC and TF during 21-day storage. Gerailoo and Ghasemnezhad (2011) suggested that SA treatment stimulated bioactive compounds including antioxidants and antioxidant enzymes. In addition, the use of SA induced TF in grape berries by increasing phenylalanine ammonia-lyase activity in the polyphenol synthesis pathway (Sarikhani *et al.*, 2010).

Razavi *et al.* (2014) applied SA to peach fruit and obserced a maintenance of firmness by inducing bioactive compounds such as antioxidant capacity and TF content. Our results show 676.290 µg acid gal g^{-1} f.w for 'Golden Delicious' variety and 816.613 µg acid gal g^{-1} f.w for 'Red Chief' variety. The results obtained in previous works suggest similar results (Khanizadeh *et al.* 2008), although there are differences in the units reported and the spectrophotometric standards used make direct comparison difficult. Differences in relation to the present study can be attributed to geographical location, sampling methods used (Vieira *et al.*, 2011; Drogoudi and Pantelidis, 2011; Quitral *et al.*, 2013 and Wang *et al.*, 2015) and most importantly crop variation. For red apples such as 'Fuji' found AC from 479.50 early 'Fuji' to 636.71 Mol TEAC/100g FP in 'Fuji' Suprema (Vieira *et al.*, 2011).

As for Ca concentrations, it was observed in our data that 70 mM is optimal for increasing and maintaining quality parameters. Previous studies confirmed that foliar Ca treatments on apple cv 'Golden Smoothee' increased K levels in fruit pulp, TA at harvest after storage, as well as TSS content. TA and TSS are positively correlated with K content (Casero *et al.*, 2004). Higher sprayed Ca content was positively correlated with higher fruit firmness at harvest Casero *et al.* (2013), which suggest that increasing applications from the second half of the cycle raises fruit Ca levels. Furthermore, it had no effect on fruit acidity.

On the other hand, treatment with 0.15% foliar Ca in 'Golden Smoothee' apple and an increase in K in the fruit pulp increased titratable acidity at harvest and after storage, as well as TSS content (Casero *et al.*, 2013). Besides, TA and TSS were positively correlated with K content (Casero *et al.*, 2004).

Calcium improves fruit firmness by cross-linking pectic polysaccharide chains by virtue of its divalent ionic character. Calcium binding to cell wall components can also reduce the accessibility of cell wall degrading enzymes to their substrates (Vicente *et al.*, 2009).

After storage, fruit TSS was not affected by spray measures, averaging 13.4% and 13.5% °Brix in 2008 and 2009, respectively. Apples sprayed with Ca were firmer and contained more acids than those in the control plots. The efficiency of Ca sprays in maintaining fruit firmness and acidity during storage did not depend on the fertiliser tested (Wójcik and Mieczysław, 2013).

Increased fruit Ca content was more successfully achieved with foliar Ca applications than with soil applications in apple orchards. Moreover, Ca applications could increase antioxidant capacity, including total phenol and ascorbic acid content (Torres *et al*., 2017).

Jan *et al*. (2016) observed, in Red Delicious apples, that sugar content decreased significantly with increasing $CaCl_2$ concentration compared to untreated fruit. Generally, in fruits, sugars tend to increase with ripening, which could be due to decomposition/hydrolysis of starch, delaying the changes associated with ripening and senescence in fruits (Gupta *et al*., 2011). Therefore, the decrease in sugars with increasing $CaCl_2$ concentration was probably due to slow senescence and decreased water loss, calcium pectate formation leading to increased cell wall stiffness and improved turgor pressure.

Wang *et al*. (2015), in their research work, conclude that in addition to P, Ca, and B, K, are the key elements that are associated with apple fruit quality.

An increase in K increased fruit TA at harvest as well as TSS content at harvest. TA and TSS are associated with good eating quality and these parameters are positively correlated with K content (Casero *et al*., 2004). TSS content should be maintained at an average of at least 11.0 °Brix for red apples and at least 12 °Brix for yellow and green apples (NMX-FF-061-2003). Our results are above the parameters of the Mexican standard, being 12.4 °Brix for the 'Red Chief' variety and 12.3 °Brix for the 'Golden Delicious' variety which may indicate a decrease of K in the plant tissues.

It has also been found that K promotes assimilation in apple trees, accelerating the transport of starch and sugars, which leads to a higher content of soluble sugars in the fruit (Milford *et al*., 2000). Sufficient K levels in the leaf were also found to increase the level of sugars in the fruit, improving quality, surface colour, aroma, flavour and storage (Zhang *et al*., 1997).

While Co^{+2} ion blocks ACC-oxidase acting on ACC (1-aminocyclopropane-1-carboxylic acid) which is oxidised and cleaved to ethylene with release of CO_2 (Jordan and Casaretto, 2006). Ethylene is known to control the rate of ripening, shelf-life and most ripening events in fruit (Bouzayen *et al*., 2010). Increased respiration is accompanied by a similar increase in ethylene levels that coordinate and synchronise the ripening process, causing irreversible changes in firmness (Omboki *et al*., 2015).

Co excess in sugarcane increased the activity of SOD, the enzyme responsible for oxygen (O_2) oxidation-reduction (Sinha and Chatterjee, 2014). The activities of POX and APX also increased indicating that there was an increased generation of ROS. These enzymes are responsible for neutralising H_2O_2 subsequently produced by SOD activity. The increase in POX may result in a high content of phenols in the tissues. The concentration of sugars and starch was also increased.

In cowpea bean, Gad *et al*. (2013) observed that with the addition of Co, the amount of TSS increased, improving quality. Similar results were registered in peanuts (Gad, 2012). Gad and Abdel-Moez (2011) found that applied Co levels showed a favourable effect on TSS, TA, and TF, as well as, vitamin C in broccoli heads. The addition of Co to tomato provided the highest TSS, vitamin C and decreased TA (Gad and Kandil, 2010).

Conclusions

It was found that to improve fruit finishing characteristics in apple such as weight, colour, diameter, firmness and percentage of fruit juice, a concentration of Ca 70.00 mM, Mo 0.300 mM, K 50.00 mM and SA 1.000 mM, as a whole, is suggested to positively influence these variables in 'Golden Delicious' variety. In addition, the variables positively affected by the factors in order of importance were colour, juice percentage, weight, diameter, juice density and firmness at Ca 92.11 mM, K 50.00 mM, SA 1 000 mM and Co 0.149 mM concentrations for the 'Red Chef' variety.

In terms of fruit quality parameters, the factors with the greatest effect in order of importance were SA 1.0 mM, K 58.90 mM, Mo 0.516 mM, Ca 70.00 mM and Co 8.248 mM, to positively favour and in order of importance the variables; TSS/acidity ratio, TSS, antioxidant capacity, total phenols, and titratable acidity in 'Golden Delicious' apple. In addition, the factors Ca 70.00 mM, K 58.79 mM and SA 1.0 mM, with the variables; titratable acidity, TSS/acidity ratio, total phenols, TSS and antioxidant capacity finally contribute to an optimal development of apple fruit quality for each variety.

Authors' Contributions

Conceptualization: JMSP; Methodology: JCOM, LCNM, RMYM; Validation: JMSP, JCOM; Formal analysis: JMSP, RPL; Investigation: JCOM, LCNM, RMYM; Data curation: JMSP, RPL; Funding acquisition: JMSP, ES, RPL; Project administration: JMSP; Writing: JCOM, ES; Review and editing: JCOM, JMSP, ES, LCNM; All authors read and approved the final manuscript

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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