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Research Article

Salicylic acid and nutrient immersion to maintain apple quality and bioactive compounds in postharvest

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Abstract

The world production of apples in the 2019 cycle reached 7´620,288 tonnes. For marketing purposes and to supply the demand, apple fruits need to be stored for different periods under refrigerated conditions. However, in the market, the shelf life of the fruit is short, the quality decreases in postharvest due to the dynamic changes of its physicochemical properties, which cannot be stopped, but can be slowed down to improve its shelf life. Postharvest treatments by immersing apple fruit in salicylic acid (SA) and nutrients are an innovative technological alternative to maintain their quality. In this study, 5 concentrations were tested for the immersion of apple fruits cv 'Golden Delicious', using a 56 factorial arrangement delimited to 25 treatments, using the Taguchi L25 structure: SA 0 - 1.440 mM, potassium (K) 0 - 2.250, calcium (Ca) 0 - 31.500 mM, cobalt (Co) 0 - 0.180 mM, molybdenum (Mo) 0 - 0.0900 mM and magnesium (Mg) 0 - 0.0900 mM. The study was conducted in the municipality of Cuauhtémoc, Chihuahua, Mexico. After 7 months of storage and 13 days of shelf life, the combination of K, Ca, SA and Co with the appropriate concentration values can maintain the quality variables and bioactive compounds at the desired optimum. It is concluded that the quality variables; firmness, juice percentage, juice density, titratable acidity and total soluble solids and the bioactive compounds; total phenols and antioxidant capacity can be maintained at the desired optimum.

Keywords: controlled atmosphere; chilling; factors; *Malus domestica*; postharvest

Introduction

World apple production in the 2019 cycle reached 7'620,288 tonnes. Mexico contributed 761,483 t (FAO, 2020). To supply the demand, apple fruits need to be stored under refrigerated conditions and different periods for marketing purposes (Cepeda *et al.*, 2014). Postharvest changes in fresh fruit cannot be stopped, but they can be slowed down to improve shelf life. Postharvest treatments play an important role in extending the storage and shelf life of perishable horticultural products (El-Ramady *et al.*, 2018).

Pre-storage treatments through immersion of fruits in Ca (Conway *et al.*, 2002), SA (Supapvanich, 2015) and Mg with Ca (Farag and Nagy, 2012) in postharvest, emerge as a technological and innovative

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alternative to maintain quality in apples. Quality is considered as a dynamic synthesis of physicochemical properties of fruits, and has been boosted by advances in postharvest physiology and technology (Kyriacou and Rouphael, 2017). Agricultural and postharvest practices contribute to flavour, and it is believed that the flavour quality of many fresh fruits available to consumers has deteriorated (Bartoshuk and Klee, 2013).

An important decision to ensure high fruit quality is the time of harvest. Early or late harvesting can lead to several negative aspects that decrease fruit quality (Vanoli and Buccheri, 2012). The degree of fruit ripening influences the production of volatile flavour compounds during storage and shows that harvesting too early or too late can negatively affect fruit yield and flavour development (Salas-Salazar *et al.*, 2011). Within the same orchard, variability among trees can influence final product quality, with fruit load and distribution levels being important, which can severely affect fruit quality and maturity (Serra *et al.*, 2016).

In the market, the shelf life of apples is short, due to their flimsy skin, moisture loss and high sensitivity to chilling. Physical damage to the skin of the fruit such as shrinkage of the top of the fruit, pitting, brown spots and rotting are the main problems that limit its acceptability for consumption and shelf life (Supapvanich *et al.*, 2018). The characteristics that determine apple quality can be measured or graded (Musacchi and Serra, 2018). 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.

The relationship between apple tree nutrition and fruit quality is important, just as the proper balance of nutrients is essential to maintain fruit quality and shelf life. Fruit colour, size, bitter pit, internal breakdown, and watery core are often the commercially important attributes that fruit growers wish to predict. SA and among the nutrients Ca, K, Mg, Co and Mo, are considered to have the most notable influence (Casero *et al.*, 2004).

SA, as a plant hormone is considered a safe compound for postharvest use (Asghari and Aghdam, 2010). It's used to maintain postharvest quality and delay fruit ripening (Supapvanich and Promyou. 2013). In addition, SA can strengthen fruit tissue structure by maintaining the pectin structure (Promyou and Supapvancih, 2016). In wax apple (Supapvanich *et al.*, 2018) showed that immersion in SA at a concentration of 0.5 mM, flesh firmness was maintained, and antioxidant activity and certain secondary metabolites were increased during storage.

Ca and K are important in the water balance, Ca forms part of the cell membrane and is stored between the cell wall and the middle sheet, where it interacts with peptidic acid to form calcium pectate, providing stability for its integrity. Equally important, it intervenes 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). Cepeda-Castañeda *et al.* (2014), reported that immersions of apples in 4% $CaCl_2$ increase Ca content, which favours the delay of firmness and weight loss, allowing fruit storage. K influences the permeability of cell membranes and tissue hydration, due to its high mobility in the phloem and xylem, it is important in the transport of solutes, the distribution of assimilates, and the synthesis of polyphenols responsible for colour and aroma. Besides, K positively affects the size, firmness, total soluble solids (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 (Musacchi and Serra, 2018).

Mg is a bivalent cation as Ca^{2+} and is likely to be bound between pectic substances within the cell wall or bound between polar heads in the plasma membrane. Therefore, it may maintain the integrity of the cell wall and plasma membrane (Farag and Nagy, 2012). Some apple cultivars, such as 'Golden Delicious' are very susceptible to Mg deficiency, as it can reduce productivity and fruit quality.

Co^{2+} ion is an inhibitor of the ethylene biosynthesis pathway (Lau and Yang, 1976). Few enzymes contain Mo, it acts as an enzyme co-factor, has both structural and catalytic functions and has direct implications in redox reactions (Nautiyal and Chatterjee, 2004).

Previous work has been done on the application and dosage of SA and nutrients in post-harvest immersion (Supapvanich *et al.* 2018). Their use can be a safe and reliable alternative. However, there is little information on their combined application in apple. Therefore, the aim of this work was to evaluate the effects of SA and nutrient immersion in combination to maintain the quality and improve bioactive compounds of apple cv 'Golden Delicious' during postharvest storage.

Materials and Methods

Experimental area and treatments

The research work was carried out in the 2018 cycle. It was carried out in the orchard and cold storage plant "La Campana", owned by Mr. Abram Olfert, located in the Mennonite field number 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". Finishing and fruit quality analyses were carried out in the soil laboratory of the Faculty of Agrotechnological Sciences of the Universidad Autónoma de Chihuahua.

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

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

Levels	Factors mM					
	K	Ca	Co	Mo	SA	Mg
0	0.0000	0.000	0.000	0.0000	0.000	0.0000
1	0.1125	1.575	0.009	0.0045	0.072	0.0045
5	0.5625	7.875	0.045	0.0225	0.360	0.0225
10	1.1250	15.750	0.090	0.0450	0.720	0.0450
20	2.2500	31.500	0.180	0.0900	1.440	0.0900
Simple average	1.1250	15.750	0.090	0.045	0.720	0.0450
Stock solution mM	300	500	50	100	4.5	50

Sources: Fainal K^{MR} (K, 46.5%); $CaCl_2$ (Ca, 36.11%); $CoCl_2$ (Co, 24.8%), Prosimol^{MR} (Mo, 39.0%); salicylic acid (SA, 99.7%) and $MgSO_4$ (Mg, 16.3%)

For the selection of apple fruit at harvest, special care was taken in the field to obtain 32 fruits of commercial quality per treatment, without any physical damage or visible diseases. For each treatment, 5 L of water were added to a 20 L container, the amounts of stock solution indicated in Table 2 were added, and the solution was made up to 10 L by adding water and shaking. The 32 fruits were immersed and shaken manually for 10 min. At the end of this time, they were removed and left at room temperature for 5 min to drain the excess water. They were then placed in perforated plastic bags in groups of 8 for each repetition, for storage in a controlled atmosphere for a period of 7 months.

Once the storage process was finished, the fruits were taken to the laboratory and kept at room temperature to simulate the shelf life. The quality of the fruits was evaluated 1, 5, 9 and 13 days after harvest, using 5 fruits for each repetition to obtain colour, firmness, total soluble solids (TSS), juice density, juice percentage and titratable acidity (TA). The remaining 3 fruits were used to determine the biological compounds: total phenols (TF) and antioxidant capacity (AC).

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

Treatment	Fainal K	$CaCl_2$	$CoCl_2$	Prosimol	SA	$MgSO_4$
1	0.0000	0.000	0.000	0.0000	0.000	0.0000
2	0.0000	1.575	0.009	0.0045	0.072	0.0045
3	0.0000	7.875	0.045	0.0225	0.360	0.0225
4	0.0000	15.750	0.090	0.0450	0.720	0.0450
5	0.0000	31.500	0.180	0.0900	1.440	0.0900
6	0.1125	0.000	0.009	0.0225	0.720	0.0900
7	0.1125	1.575	0.045	0.0450	1.440	0.0000
8	0.1125	7.875	0.090	0.0900	0.000	0.0045
9	0.1125	15.750	0.180	0.0000	0.072	0.0225
10	0.1125	31.500	0.000	0.0045	0.360	0.0450
11	0.5625	0.000	0.045	0.0900	0.072	0.0450
12	0.5625	1.575	0.090	0.0000	0.360	0.0900
13	0.5625	7.875	0.180	0.0045	0.720	0.0000
14	0.5625	15.750	0.000	0.0225	1.440	0.0045
15	0.5625	31.500	0.009	0.0450	0.000	0.0225
16	1.1250	0.000	0.090	0.0045	1.440	0.0225
17	1.1250	1.575	0.180	0.0225	0.000	0.0450
18	1.1250	7.875	0.000	0.0450	0.072	0.0900
19	1.1250	15.750	0.009	0.0900	0.360	0.0000
20	1.1250	31.500	0.045	0.0000	0.720	0.0045
21	2.2500	0.000	0.180	0.0450	0.360	0.0045
22	2.2500	1.575	0.000	0.0900	0.720	0.0225
23	2.2500	7.875	0.009	0.0000	1.440	0.0450
24	2.2500	15.750	0.045	0.0045	0.000	0.0900
25	2.2500	31.500	0.090	0.0225	0.072	0.0000

Each treatment was volumetrically diluted with water to 10 L of solution.

Fruit quality

To obtain colour in percentage (%), the scale developed by Soto *et al.* (2001) was used. Two measures of colour per fruit, being two intermediates in terms of colour, considering six categories for 'Golden Delicious': 1) green; 2) rough green rough lenticels; 3) waxy green; 4) transition to yellow; 5) whitish yellow (yellowish); and 6) strongly yellow with a tendency towards orange. The colour scale was expressed as a percentage.

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 the colour was measured, the peel was removed for measurement and the two readings were averaged.

For the determination of TSS in °Brix, a Red Rooster 90681 refractometer was used, with a scale of 0.0 to 32.0 °Brix. Two segments were extracted from each fruit (one for each pressure test), weighed and taken to the juice extractor, and a few drops of the sample obtained were deposited on the prism of the refractometer previously calibrated with distilled water. The extract obtained was poured into a graduated cylinder. It was 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.

The titratable acidity in % malic acid was obtained using 10 mL of the same juice used to obtain TSS, 6 drops of 1% phenolphthalein 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 equivalent of malic acid.

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

Bioactive compounds

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 (g GA g^{-1}). 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 antioxidant capacity analysis according to the methodology of Brand-Williams *et al.* (1995), with slight modifications. 2.8 ml of freshly prepared 0.1 mM 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, 80% methanol was substituted for the extract 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 where the factors and the simple average for each of them were specified. 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 the most influence on each variable. Data were analysed for each of the four dates assessed.

Results and Discussion

Table 3 shows the result of the statistical analysis at the first day of shelf life, with the frequency of signs for the eigenvectors present. Table 4 shows the selection of factors and variables on the first day of shelf life. The results show that the most important factors selected were; SA (35), Ca (35) and K (30). While the variables selected were; juice percentage (23), AC (20) and firmness (19). The selection was also carried out for 5, 9 and 13 days, the results are shown in Tables S1 to S6.

Table 3. Factors, fruit quality and bioactive compounds of apple, stored in a controlled atmosphere for 7 months and left on the shelf for 1 day

Eigenvalues	Factors / simple average [mM]							
	K **1.125**[S]	Ca **15.75**	Co **0.090**	Mo **0.045**	SA **0.720**	Mg **0.045**	Eigenvectors Total	Prop. +/-
	Colour[U] µ 60.00 (56.88 – 62.92%) R^2 0.7681 C.V. 4.67							
20.38[T]	--[V]	+++					5	3 / 2
-7.52	++	++	---				7	4 / 3
-21.19	++				+++		5	5 / 0
Freq.	6[W]	5	3	0	3	0	17[Y]	12 / 5
[mM][X]							Selection ≥ 3	
	Firmness µ 10.65 (9.38 – 12.05 lb in^2) R^2 0.9192 C.V. 6.23							
45.18	+++				--		5	3 / 2
18.56		+++			++	--	7	5 / 2
-19.15		+++	++			++	7	7 / 0
Freq.	3	6	2	0	4	4	19	15 / 4
[mM]	0.172	15.43			1.035	0.039	Selection ≥ 4	
	Total soluble solids µ 13.8 (12.0– 15.0 °Brix) R^2 0.9034 C.V. 5.51							
30.01	++++						4	4 / 0
20.52			++	--	+++		7	5 / 2
-13.69		++	+++				5	5 / 0
Freq.	4	2	5	2	3	0	16	14 / 2
[mM]							Selection ≥ 3	
	Titratable acidity µ 0.3894 (0.2546 – 0.4757% malic acid) R^2 0.9325 C.V. 10.67							
62.51					+++		3	3 / 0
22.88	+++	++	--				7	5 / 2
-54.85		+++	++				5	5 / 0
Freq.	3	5	4	0	3	0	15	13 / 2
[mM]							Selection ≥ 3	
	Rel. Sugar Acidity µ 35.79 (29.43 – 51.06 °Brix / malic acid) R^2 0.9383 C.V. 10.69							
69.95		+++					3	3 / 0
-77.37				++	+++		5	5 / 0
Freq.	0	3	0	2	3	0	8	8 / 0
[mM]							Selection ≥ 2	
	Juice density µ 3.27 (2.51 – 4.95 g ml^{-1}) R^2 0.9768 C.V. 9.91							
68.16	+++		++	--			7	5 / 2
45.56				++	+++		5	5 / 0
-52.72		++	++	++			6	6 / 0
Freq.	3	2	4	6	3	0	18	16 / 2
[mM]							Selection ≥ 4	
	Percentage of juice µ 81.85 (38.89 – 91.30%) R^2 0.5101 C.V. 34.26							
14.83		--		++	++		6	4 / 2
6.55		++		+++			5	5 / 0
-19.54			++		+++		5	5 / 0
-38.90	+++	++	--				7	5 / 2
Freq.	3	6	4	5	5	0	23	19 / 4
[mM]		1.125	15.75		0.045	0.720		Selection ≥ 5

	Total phenols µ 480.14 (363.38 – 637.58 µg gal acid gr^{-1} p.f) R^2 0.8029 C.V. 6.62							
84.37	+++				+++		6	6 / 0
53.10	--			++	+++		7	5 / 2
-63.19		+++	++				5	5 / 0
Freq.	5	3	2	2	6	0	18	16 / 2
[mM]							Selection ≥ 4	
	Antioxidant capacity µ 3.051 (1.826 – 4.594 mg trolox g^{-1} p.f.) R^2 0.9029 C.V. 7.07							
79.16				++	+++		5	5 / 0
35.81	+++			--			5	3 / 2
-36.46				+++	--		5	3 / 2
-64.82		+++				++	5	5 / 0
Freq.	3 L,C; SA[W]	3 L,C; SA	0	7	5 L, C	2	20	16 / 4
[mM]	1.320**[X]	12.72**			1.048**		Selection ≥ 4	
	Summary						Total Prop.+/-	
Subtotal	30	35	24	24	35	6	154	
Selection	0 / 9	2 / 9	0 / 9	2 / 9	3 / 9	0 / 9	Variables 3 / 9	
Prop. +/-	26 / 4	33 / 2	17 / 7	18 / 6	31 / 4	4 / 2	129[Z] / 25	
[ ]	1.320	15.75			1.048		Selec. ≥26 (19)	

[S]Simple mean factor levels; [T]Eigenvalues expressed as a percentage of the mean of the response variable; [U]Range in parentheses corresponds to the predicted values from the simple mean; [V]Each sign corresponds to multiples of 0.25 rounded to the nearest quarter; [W]linear regression response type L, quadratic C and factor interaction; [X]Optimal value of the predicted factors and probability: significant * (0. 05 ≤ Pr ≤ 0.01), highly significant ** (Pr <0.01), otherwise not significant; [Y]Total observed frequency for that variable, [Z]Total frequency for the set of variables, factors with a subtotal equal to or greater than 20% are selected while variables greater than or equal to 15% are selected

Table 4. Selection factors, quality variables and bioactive compounds of apples, stored in a controlled atmosphere for 7 months and left on shelf for 1 day

Eigenvalues	Factors / simple average [mM]						Eigenvectors	
	K **1.125**[S]	Ca **15.75**	Co **0.090**	Mo **0.045**	SA **0.720**	Mg **0.045**	Total	Prop. +/-
	Colour[U] µ 60.00 (56.88 – 62.92%) R^2 0.7681 C.V. 4.67							
Freq.	6[W]	5	3	0	3	0	17[Y]	12 / 5
[mM][X]							Selection ≥ 3	
	Firmness µ 10.65 (9.38 – 12.05 lb in^2) R^2 0.9192 C.V. 6.23							
Freq.	3	6	2	0	4	4	19	15 / 4
[mM]	0.172	15.43	0.103	0.035	1.035	0.039	Selection ≥ 4	
	Total soluble solids µ 13.8 (12.0– 15.0 °Brix) R^2 0.9034 C.V. 5.51							
Freq.	4	2	5	2	3	0	16	14 / 2
[mM]							Selection ≥ 3	
	Titratable acidity µ 0.3894 (0.2546 – 0.4757% malic acid) R^2 0.9325 C.V. 10.67							
Freq.	3	5	4	0	3	0	15	13 / 2
[mM]							Selection ≥ 3	
	Rel. TSS/acidity µ 35.79 (29.43 – 51.06 °Brix/ malic acid) R^2 0.9383 C.V. 10.69							
Freq.	0	3	0	2	3	0	8	8 / 0
[mM]							Selection ≥ 2	
	Juice density µ 3.27 (2.51 – 4.95 g ml^{-1}) R^2 0.9768 C.V. 9.91							
Freq.	3	2	4	6	3	0	18	16 / 2
[mM]							Selection ≥ 4	

	Percentage of juice μ 81.85 (38.89 – 91.30%) R^2 0.5101 C.V. 34.26							
Freq.	3	6	4	5	5	0	23	19 / 4
[mM]	1.125	15.75	0.090	0.045	0.720	0.045	Selection ≥ 5	
	Total phenols μ 480.14 (363.38 – 637.58 μg gal acid gr^{-1} p.f) R^2 0.8029 C.V. 6.62							
Freq.	5	3	2	2	6	0	18	16 / 2
[mM]							Selection ≥ 4	
	Antioxidant capacity μ 3.051 (1.826 – 4.594 mg trolox g^{-1} p.f.) R^2 0.9029 C.V. 7.07							
Freq.	3 L,C; SA^{W}	3 L,C; SA	0	7	5 L, C	2	20	16 / 4
[mM]	1.320**^{X}	12.72**			1.048**		Selection ≥ 4	
	Summary						Total Prop.+/-	
Subtotal	30	35	24	24	35	6	154	
Selection	0 / 9	2 / 9	0 / 9	2 / 9	3 / 9	0 / 9	Variables 3 / 9	
Prop. +/-	26 / 4	33 / 2	17 / 7	18 / 6	31 / 4	4 / 2	129^{Z} / 25	
[ ]	1.320	15.75			1.048		Selec. ≥26 (19)	

^{S}Simple mean factor levels; ^{U}Range in brackets corresponds to the predicted values from the simple mean; ^{W}linear regression response type L, quadratic C and factor interaction; ^{X}Optimal value of the predicted factors and probability: significant * (0.05 ≤ Pr ≤ 0. 01), highly significant ** (Pr <0.01), otherwise not significant; ^{Y}Total observed frequency for that variable, ^{Z}Total frequency for the set of variables, those factors with a subtotal equal or greater than 20% are selected while variables greater or equal to 15% are selected

Fruit quality

The sum of the eigenvector weighting values in the statistical analysis of the four evaluated shelf-life dates, K, Ca, SA and Co, were the selected factors in order of importance, as well as the highest observed application concentration values in mM, are presented in Table 5.

Table 5. Weighting of eigenvectors by day of shelf life, to select factors and application concentrations

Factor	Shelf days and Eingenvectors [mM]			
	1	5	9	13
K	30 [1.320]	41 [1.483]	38 [1.125]	36 [1.285]
Ca	35 [15.75]	35 [21.17]	29 [15.75]	34 [15.00]
SA	35 [1.048]	31 [1.047]	35 [0.720]	23 [1.196]
Co			32 [0.090]	

The weighting of eigenvectors for the selection of the quality variables, and to maintain them in the desired optimum, were in order of importance, with the maximum values of the original data range; firmness in lb in^2 (12.05 at day one, 11.56 at five days and 11.38 at thirteen days); juice percentage (91.30 at day one and 80.31 at thirteen days); titratable acidity with 0.4442 % malic acid at day five. On the other hand, with the minimum values of the original data range were; juice density 1.81 g ml^{-1} at day nine); and 12.40 °Brix TSS at day five, data shown in Table 6.

Table 6. Weighting of eigenvectors by day of shelf-life for variable selection, mean and original range

Variable	Weighting of eigenvalues				Value of the mean and (original range)			
	1	5	9	13	1	5	9	13
Firmness	19	20		17	10.65 (9.38-12.05)	10.49 (9.84-11.56)		10.05 (8.87-11.38)
% Juice	23			17	81.85 (38.89-91.30)			
			26					
Acidity titratable		24				0.3583 (0.2881-0.4442)		
TSS		21				13.80 (12.40-15.40)		
Total phenols				20				434.097 (292.42-600.48)
Antioxidant capacity	20				3.051 (1.83-4.59)			

Firmness (lb in^2), juice percentage (%), juice density (g ml^{-1}), titratable acidity (% malic acid), TSS (°Brix), total phenols (µg gal acid g^{-1} p.f.) and antioxidant capacity (mg trolox g^{-1} p.f.).

Previous studies have shown that SA dives, at an adequate concentration, maintain postharvest quality during storage (Promyou and Supapvanich, 2016). For apple fruit to be accepted in some markets, firmness must be at least 62.3 N (Delong *et al.*, 2000). Our results of postharvest immersion of apple fruit cv 'Golden Delicious' with the combination of K, Ca and SA at concentrations of 1.285 mM, 15.00 mM and 1.196 mM respectively, 13 days after controlled atmosphere storage for 7 months, can maintain firmness at 11.38 lb in^2, and are similar to those reported. Supapvanich *et al.* (2018), showed that immersion of wax apple fruits in SA at a concentration of 0.5 mM maintained firmness, being higher with an immersion at 1.0 mM. These results confirms that SA stimulates phenylalanine ammonia lyase (PAL) activity (Dong *et al.*, 2010), induces cell swelling and inhibits cell wall hydrolase activities, and cellulase, polygalacturonase (PG), lipoxygenase (LOX) and pectin methylesterase (PME) enzymes that degrade the membrane (Asghari and Aghdam, 2010). Similar results in rambutan (Manganaris *et al.*, 2007) and in peach fruits, which, maintained the firmness (Supapvanich 2015). Thus, exogenous application of SA improves defence mechanisms and antioxidant production in fruits during storage, leading to a decrease in cell membrane lipid peroxidation (Wei *et al.*, 2011).

Similar work to ours using different combinations of treatments in apple found a significant increase in firmness when combining $CaCl_2$ (1% and 2%) and $MgCl_2$ (1% and 2%) salts to maintain higher tissue firmness (Farag and Nagy, 2012). Shafiee *et al.* (2010), showed that strawberry dives treated postharvest with 2.0 mM SA combined with 1.0% $CaCl_2$ after cold storage had increased firmness. In papaya, a concentration of 2.0 mM is necessary to delay the loss of firmness (Promyou and Supapvanich, 2016). They report that, immersion of apple in 3.5% $CaCl_2$ solutions for a period of 30 s, is sufficient to significantly reduce the Bitter pit, but with no effect on fruit firmness (Torres *et al.*, 2017). However, Cepeda-Castañeda *et al.* (2014), found that, the highest flesh firmness with a mean of 79.2 N and a high TSS/acidity ratio of 41.7 was obtained with immersions in a 6% $CaCl_2$ solution for 15 min postharvest. In plum fruit treated in $CaCl_2$ immersion at a concentration of 1.0 mM, Valero *et al.* (2002) found that fruit firmness was higher than that of control fruit in storage. On the other hand, Manganaris *et al.* (2007), in peach using three Ca sources and two immersion concentrations (62.5 and 187.5 mM Ca), report that treatment with Ca salts at 62.5 mM was as effective as the higher concentration, maintaining tissue firmness during storage.

In our study, TSS can be maintained at 12.40 °Brix, and TA with 0.4442% malic acid, which is very acceptable at 5 days after storage. If the fruit is harvested ripe, TSS slightly increases in concentration after harvest, starch (higher at harvest) is converted to sugars through hydrolysis over time (Musacchi and Serra. 2018), to aid respiration (Mesa *et al.*, 2016). Previous work showed that SA concentration in immersion did not affect TSS and TA in wax apple (Supapvanich *et al.*, 2018), in rambutan (Supapvanich 2015). Kazemi *et al.* (2011) showed that SA treatments delayed the decrease in titratable acidity during ripening of kiwifruit, similar results in pineapple (Lu *et al.*, 2011). In postharvest (Guerra and Casquero, 2010) with apple fruit treated by immersion with $CaCl_2$ at a concentration of 2%, for 30 s, after 60 days of cold storage, they report that the decrease in firmness was lower, but after 120 days of cold storage, there was no difference between treated and untreated fruit but, they were useful to retain the acidity of the fruit until 120 days of storage. Manganaris *et al.* (2007) report that the TSS and TA content of peach fruit was not influenced by postharvest Ca dives. Similar work to ours, Shafiee *et al.* (2010), applied 2.0 mM SA dives combined with 1.0% $CaCl_2$, after cold storage, , showed that postharvest treated strawberry fruit did not influence TSS and TA compared to the control. However, Bal (2016), in nectarine with exogenous application of SA, reports a gradual decrease in titratable acidity with prolonged storage.

Bioactive compounds

In our results, total phenols (TF) with the combination of K, Ca and SA at concentrations of 1.285 mM, 15.00 mM and 1.196 mM respectively, can be maintained at 600.48 µg acid gal g^{-1} FW at 13 days of shelf life, and antioxidant capacity (AC) at 4.59 mg trolox g^{-1} FW at the first day of shelf life with concentrations of 1.320 mM K, 15.75 mM Ca and 1.048 mM SA. Previous work showed that immersion of wax apple fruits in SA at a concentration of 0.5 mM enhanced the bioactive compounds than using 1.0 mM, demonstrating that their content is dose dependent (Supapvanich *et al.*, 2018). This confirms that SA stimulates phenylalanine ammonia lyase (PAL) activity and the antioxidant activities of the enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), leading to an increase in phenolic acids (Dong *et al.*, 2010), in addition to bioactive compounds, which provides health benefits (Supapvanich and Promyou. 2013). In a similar work with SA immersion, Wei *et al.* (2011) found that SA induced the maximum postharvest phenolic concentration in immersion-treated asparagus at a concentration of 1.0 mM, as well as containing a higher concentration of DPPH scavenging activity. In sweet cherry treated with SA immersion, total anthocyanin and total phenols were improved during storage (Valero *et al.*, 2011). In carnelian cherries treated in immersion with 2.0 mM SA, Dokhanieh *et al.* (2013) reported that the content of total phenols and flavonoids increased significantly during postharvest storage, while cherries treated with a concentration of 1.0 mM had higher DPPH radical scavenging capacity. In nectarine with exogenous application of SA Bal (2016) found a higher concentration of phenolic compounds and flavonoids with prolonged storage. SA at an adequate concentration maintains postharvest quality, (Promyou and Supapvanich, 2016) showed that, immersions at a concentration of 2.0 mM in papaya improved antioxidant capacity and total phenols during storage.

Conclusions

This article reports for the first time, the positive effect of the combination of K, SA, and Ca postharvest immersion with the appropriate concentration: K 1.285 mM, Ca 15.00 mM, SA 1.196 mM, values to maintain the quality variables and bioactive compounds in the desired optimum, for apple fruits cv 'Golden Delicious', stored in a controlled atmosphere for 7 months. It was obtained that the factors with the greatest contribution with the highest weight, through the analysis of the 4 shelf-life dates, were K, Ca, and SA in order of importance to preserve the desired quality variables (firmness, juice percentage, juice density, titratable acidity and total soluble solids) and bioactive compounds (total phenols and antioxidant capacity).

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.

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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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Salicylic acid and nutrient immersion to maintain apple quality and bioactive compounds in postharvest

Supplementary files

Table S1. Factors, fruit quality and bioactive compounds of apple, stored in a controlled atmosphere for 7 months and left on the shelf for 5 days

Eigenvalues	Factors / simple average [mM]							
	K 1.125[S]	Ca 15.75	Co 0.090	Mo 0.045	SA 0.720	Mg 0.045	Eigenvectors Tot.	Prop. +/-
	Colour[U] µ 60.72 (57.50 – 65.52%) R^2 0.9657 C.V. 1.86							
21.64[T]		+++[V]				++	5	5 / 0
-10.77	+++			--			5	3 / 2
-24.70			++		+++		5	5 / 0
Frec.	3[W]	3	2	2	3	2	15[Y]	13/ 2
[mM]							Selection ≥ 3	
	Firmness µ 10.49 (9.84 – 11.56 lb in^2) R^2 0.9885 C.V. 1.40							
17.43	+++				--		5	3 / 2
11.95			+++	++			5	5 / 0
-6.11	++				+++		5	5 / 0
-10.87			++	---			5	2 / 3
Frec.	5 C; SA[W]	0	5 C	5 C	5 C	0	20	15 / 5
[mM]	1.483[X]		0.083	0.062	0.121		Selection ≥ 4	
	Total soluble solids µ 13.80 (12.40 – 15.40 °Brix) R^2 0.9709 C.V. 3.06							
32.98	---				++		5	2 / 3
25.62	+++				++		5	5 / 0
18.11		++			++	--	6	4 / 2
-27.33		+++				++	5	5 / 0
Frec.	6 C	5 C	0	0	6 C	4 C	21	16 / 5
[mM]	0.852	12.61			1.047	0.042	Selection ≥ 4	
	Titratable acidity µ 0.3583 (0.2881 – 0.4442% malic acid) R^2 0.9527 C.V. 7.91							
34.00	---	++					5	2 / 3
18.86	++	+++	--				7	5 / 2
-10.40			++	---			5	2 / 3
-25.05	++			++	+++		7	7 / 0
Frec.	7	5		5	3	0	24	16 / 8
[mM]	0.105	21.17		0.042			Selection ≥ 5	
	Rel. Sugar Acidity µ 38.80 (32.56 – 45.12 °Brix / malic acid) R^2 0.9413 C.V. 7.35							
48.49	++				+++		5	5 / 0
17.89	++			---			5	2 / 3
-39.00		+++					3	3 / 0
Frec.	4	3	0	3	3	0	13	10 / 3
[mM]							Selection ≥ 3	
	Juice density µ 2.60 (1.84 – 3.57 g ml^{-1}) R^2 0.9582 C.V. 11.40							
64.28	+++	++					5	5 / 0
26.38				--	+++		5	3 / 2
-22.50		+++	++				5	5 / 0
Frec.	3	5	2	2	3	0	15	13 / 2

[mM]							Selection ≥ 3	
	Percentage of juice µ 83.14 (74.23 – 91.07%) R^2 0.9705 C.V. 3.55							
34.79	---	++					5	2 / 3
-8.99			+++	++			5	5 / 0
-14.44	++	++				++	6	6 / 0
Frec.	5	4	3	2	0	2	16	13 / 3
[mM]							Selection ≥ 3	
	Total phenols µ 475.710 (361.774 – 713.387 µg gal acid gr^{-1} p.f) R^2 0.7993 C.V. 7.34							
30.85				+++	++		5	5 / 0
-39.24		+++			+++		6	6 / 0
-58.48	+++	++		++			7	7 / 0
Frec.	3	5	0	5	5	0	18	18 / 0
[mM]							Selection ≥ 4	
	Antioxidant capacity µ 3.351 (0.996 – 4.070 mg trolox g^{-1} p.f.) R^2 0.9232 CV. 6.16							
144.24	++				+++		5	5 / 0
48.80	+++	++	--				7	5 / 2
-113.98		+++					3	3 / 0
Frec.	5	5	2	0	3	0	15	13 / 2
[mM]							Selection ≥ 3	
	Summary						Total prop. +/-	
Subtotal	41	35	18	24	31	8	157	
Selection	3 / 9	1 / 9	1 / 9	1 / 9	2 / 9	1 / 9	Variables 3 / 9	
Prop. +/-	32 / 9	35 / 0	14 / 4	11 / 13	29/ 2	6 / 2	127[Z] / 30	
[mM]	1.483	21.17			1.047		Selec.≥25 (19)	

[S]Simple mean factor levels; [T]Eigenvalues expressed as a percentage of the mean of the response variable; [U]Range in parentheses corresponds to the predicted values from the simple mean; [V]Each sign corresponds to multiples of 0.25 rounded to the nearest quarter; [W]linear regression response type L, quadratic C and factor interaction; [X]Optimal value of the predicted factors and probability: significant * (0. 05 ≤ Pr ≤ 0.01), highly significant ** (Pr <0.01), otherwise not significant; [Y]Total observed frequency for that variable, [Z]Total frequency for the set of variables, factors with a subtotal equal to or greater than 20% are selected while variables greater than or equal to 15% are selected

Table S2. Selection of factors, quality variables and bioactive compounds of apples, stored in a controlled atmosphere for 7 months and left on the shelf for 5 days

Eigenvalues	Factors / simple average [mM]						Eigenvectors Tot. Prop. +/-	
	K **1.125**[S]	Ca **15.75**	Co **0.090**	Mo **0.045**	SA **0.720**	Mg **0.045**		
	Colour[U] µ 60.72 (57.50 – 65.52%) R^2 0.9657 C.V. 1.86							
Frec.	3[W]	3	2	2	3	2	15[Y]	13/ 2
[mM]							Selection ≥ 3	
	Firmness µ 10.49 (9.84 – 11.56 lb in^2) R^2 0.9885 C.V. 1.40							
Frec.	5 C; SA	0	5 C	5 C	5 C	0	20	15 / 5
[mM]	1.483[X]		0.083	0.062	0.121		Selection ≥ 4	
	Total soluble solids µ 13.80 (12.40 – 15.40 °Brix) R^2 0.9709 C.V. 3.06							
Frec.	6 C	5 C	0	0	6 C	4 C	21	16 / 5
[mM]	0.852	12.61			1.047	0.042	Selection ≥ 4	
	Titratable acidity µ 0.3583 (0.2881 – 0.4442% malic acid) R^2 0.9527 C.V. 7.91							
Frec.	7	5	4	5	3	0	24	16 / 8
[mM]	0.105	21.17	0.099	0.042	0.736	0.035	Selection ≥ 5	
	Rel. Sugar Acidity µ 38.80 (32.56 – 45.12 °Brix / malic acid) R^2 0.9413 C.V. 7.35							
Frec.	4	3	0	3	3	0	13	10 / 3
[mM]							Selection ≥ 3	
	Juice density µ 2.60 (1.84 – 3.57 g ml^{-1}) R^2 0.9582 C.V. 11.40							
Frec.	3	5	2	2	3	0	15	13 / 2
[mM]							Selection ≥ 3	
	Percentage of juice µ 83.14 (74.23 – 91.07%) R^2 0.9705 C.V. 3.55							
Frec.	5	4	3	2	0	2	16	13 / 3
[mM]							Selection ≥ 3	
	Total phenols µ 475.710 (361.774 – 713.387 µg gal acid gr^{-1} p.f) R^2 0.7993 C.V. 7.34							
Frec.	3	5	0	5	5	0	18	18 / 0
[mM]							Selection ≥ 4	
	Antioxidant capacity µ 3.351 (0.996 – 4.070 mg trolox g^{-1} p.f.) R^2 0.9232 CV. 6.16							
Frec.	5	5	2	0	3	0	15	13 / 2
[mM]							Selection ≥ 3	
	Summary						Total prop. +/-	
Subtotal	41	35	18	24	31	8	157	
Selection	3 / 9	1 / 9	1 / 9	1 / 9	2 / 9	1 / 9	Variables 3 / 9	
Prop. +/-	32 / 9	35 / 0	14 / 4	11 / 13	29/ 2	6 / 2	127[Z] / 30	
[mM]	1.483	21.17			1.047		Selec.≥ 25 (19)	

[S]Simple mean factor levels; [U]Range in brackets corresponds to the predicted values from the simple mean; [W]linear regression response type L, quadratic C and factor interaction; [X]Optimal value of the predicted factors and probability: significant * (0.05 ≤ Pr ≤ 0. 01), highly significant ** (Pr <0.01), otherwise not significant; [Y]Total observed frequency for that variable, [Z]Total frequency for the set of variables, those factors with a subtotal equal or greater than 20% are selected while variables greater or equal to 15% are selected

Table S3. Factors, fruit quality and bioactive compounds of apple, stored in a controlled atmosphere for 7 months and left on the shelf for 9 days

Eigenvalues	Factors / simple average [mM]							
	K **1.125**[S]	Ca **15.75**	Co **0.090**	Mo **0.045**	SA **0.720**	Mg **0.045**	Eigenvectors Tot.	Prop. +/-
	Colour[U] µ 63.76 (59.58 – 66.46%) R^2 0.8019 C.V. 4.15							
6.09[T]	--[V]		++		+++		7	5 / 2
2.77	++	--			++	++	8	6 / 2
-6.74	++	+++					5	5 / 0
Frec.	6[W]	5	2	0	5	2	20[Y]	16 / 4
[mM]							Selection ≥ 4	
	Firmness µ 10.30 (9.17 – 11.51 lb in^2) R^2 0.9685 C.V. 3.95							
78.98			++		+++		5	5 / 0
-39.52	+++	++					5	5 / 0
-66.03		+++					3	3 / 0
Frec.	3	5	2	0	3	0	13	13 / 0
[mM]							Selection ≥ 3	
	Total soluble solids µ 13.7 (12.0 – 14.6 °Brix) R^2 0.9629 C.V. 3.30							
27.07	++++						4	4 / 0
25.54					++++		4	4 / 0
-20.12		++	+++				5	5 / 0
Frec.	4	2	3	0	4	0	13	13 / 0
[mM]							Selection ≥ 3	
	Titratable acidity µ 0.3532 (0.3015 – 0.3953% malic acid) R^2 0.9710 C.V. 4.95							
56.07			++		+++		5	5 / 0
42.31	++++						4	4 / 0
-56.18		+++	++			++	7	7 / 0
Frec.	4	3	4	0	3	2	16	16 / 0
[mM]							Selection ≥ 3	
	Rel. Sugar Acidity µ 38.88 (33.45 – 46.36 °Brix / malic acid) R^2 0.9436 C.V. 7.15							
39.94		+++				++	5	5 / 0
-21.54	+++	++				--	7	5 / 2
-40.36			++	++	+++		7	7 / 0
Frec.	3	5	2	2	3	4	19	17 / 2
[mM]							Selection ≥ 4	
	Juice density µ 2.15 (1.81 – 2.64 g ml^{-1}) R^2 0.8788 C.V. 12.20							
34.6	+++			++	--		7	5 / 2
12.9	--		++	++			6	4 / 2
-8.4				+++	+++		6	6 / 0
-14.4	++		+++		++		7	7 / 0
Frec.	7	0	5	7	7	0	26	22 / 4
[mM]	1.125[X]	15.75	0.090	0.045	0.720	0.045	Selection ≥ 5	
	Percentage of juice µ 87.64 (78.08 – 95.10%) R^2 0.9149 C.V. 4.44							
18.73	--	+++					5	3 / 2
-5.82			+++		--		5	3 / 2
-13.63	++		++		+++		7	7 / 0
Frec.	4	3	5	0	5	0	17	13 / 4
[mM]							Selection ≥ 3	

	Total phenols µ 469.968 (356.939 – 590.806 µg gal acid gr^{-1} p.f) R^2 0.7977 C.V. 6.43							
32.83		+++				++	5	5 / 0
-26.36	++		++	++			6	6 / 0
-51.80			++		+++		5	5 / 0
Frec.	2	3	4	2	3	2	16	16 / 0
[mM]							Selection ≥ 3	
	Antioxidant capacity µ 2.780 (1.998 – 3.656 mg trolox g^{-1} p.f.) R^2 0.7220 C.V. 8.03							
20.38	++	+++				++	7	7 / 0
12.60	+++		++				5	5 / 0
-35.00			+++	++	++		7	7 / 0
Frec.	5	3	5	2	2	2	19	19 / 0
[mM]							Selection ≥ 4	
Summary							Total prop. + / -	
Subtotal	38	29	32	13	35	12	159	
Selection	1 / 9	0 / 9	1 / 9	1 / 9	1 / 9	0 / 9	Variables 1 / 9	
Prop. +/-	32 / 6	27 / 2	32 / 0	13 / 0	31/ 4	10 / 2	145[Z] / 14	
[mM]	1.125	15.75	0.090		0.720		Selec. ≥ 29 (22)	

[S]Simple mean factor levels; [T]Eigenvalues expressed as a percentage of the mean of the response variable; [U]Range in parentheses corresponds to the predicted values from the simple mean; [V]Each sign corresponds to multiples of 0.25 rounded to the nearest quarter; [W]linear regression response type L, quadratic C and factor interaction; [X]Optimal value of the predicted factors and probability: significant * (0. 05 ≤ Pr ≤ 0.01), highly significant ** (Pr <0.01), otherwise not significant; [Y]Total observed frequency for that variable, [Z]Total frequency for the set of variables, factors with a subtotal equal to or greater than 20% are selected while variables greater than or equal to 15% are selected

Table S4. Selection factors, quality variables and bioactive compounds of apple, stored in a controlled atmosphere for 7 months and left on shelf for 9 days

Eigenvalues	Factors / simple average [mM]							
	K **1.125**[S]	Ca **15.75**	Co **0.090**	Mo **0.045**	SA **0.720**	Mg **0.045**	Eigenvectors Tot. Prop. +/-	
	Colour[U] µ 63.76 (59.58 – 66.46%) R^2 0.8019 C.V. 4.15							
Frec.	6[W]	5	2	0	5	2	20[Y]	16 / 4
[mM]							Selection ≥ 4	
	Firmness µ 10.30 (9.17 – 11.51 lb in^2) R^2 0.9685 C.V. 3.95							
Frec.	3	5	2	0	3	0	13	13 / 0
[mM]							Selection ≥ 3	
	Total soluble solids µ 13.7 (12.0 – 14.6 ºBrix) R^2 0.9629 C.V. 3.30							
Frec.	4	2	3	0	4	0	13	13 / 0
[mM]							Selection ≥ 3	
	Titratable acidity µ 0.3532 (0.3015 – 0.3953% malic acid) R^2 0.9710 C.V. 4.95							
Frec.	4	3	4	0	3	2	16	16 / 0
[mM]							Selection ≥ 3	
	Rel. Sugar Acidity µ 38.88 (33.45 – 46.36 ºBrix / malic acid) R^2 0.9436 C.V. 7.15							
Frec.	3	5	2	2	3	4	19	17 / 2
[mM]							Selection ≥ 4	
	Juice density µ 2.15 (1.81 – 2.64 g ml^{-1}) R^2 0.8788 C.V. 12.20							
Frec.	7	0	5	7	7	0	26	22 / 4
[mM]	1.696[X]	14.62	0.078	0.064	0.205		Selection ≥ 5	
	Percentage of juice µ 87.64 (78.08 – 95.10%) R^2 0.9149 C.V. 4.44							
Frec.	4	3	5	0	5	0	17	13 / 4
[mM]							Selection ≥ 3	
	Total phenols µ 469.968 (356.939 – 590.806 µg gal acid gr^{-1} p.f) R^2 0.7977 C.V. 6.43							
Frec.	2	3	4	2	3	2	16	16 / 0
[mM]							Selection ≥ 3	
	Antioxidant capacity µ 2.780 (1.998 – 3.656 mg trolox g^{-1} p.f.) R^2 0.7220 C.V. 8.03							
Frec.	5	3	5	2	2	2	19	19 / 0
[mM]							Selection ≥ 4	
	Summary						Total prop. + / -	
Subtotal	38	29	32	13	35	12	159	
Selection	1 / 9	0 / 9	1 / 9	1 / 9	1 / 9	0 / 9	Variables 1 / 9	
Prop. +/-	32 / 6	27 / 2	32 / 0	13 / 0	31/ 4	10 / 2	145[Z] / 14	
[mM]	1.125	15.75	0.090		0.720		Selec. ≥ 29 (22)	

[S]Simple mean factor levels; [U]Range in brackets corresponds to the predicted values from the simple mean; [W]linear regression response type L, quadratic C and factor interaction; [X]Optimal value of the predicted factors and probability: significant * (0.05 ≤ Pr ≤ 0. 01), highly significant ** (Pr <0.01), otherwise not significant; [Y]Total observed frequency for that variable, [Z]Total frequency for the set of variables, those factors with a subtotal equal or greater than 20% are selected while variables greater or equal to 15% are selected

Table S5. Factors, fruit quality and bioactive compounds of apple, stored in a controlled atmosphere for 7 months and left on the shelf for 13 days

Eigenvalues	Factors / simple average [mM]						Eigenvectors Tot.	Prop. +/-
	K **1.125**^{S}	Ca **15.75**	Co **0.090**	Mo **0.045**	SA **0.720**	Mg **0.045**		
	Colour^{U} µ 66.71 (62.29 – 69.48%) R^2 0.9195 C.V. 2.69							
26.77^{T}				++	+++		5	5 / 0
8.53	++^{V}		++	---			7	4 / 3
-25.20		++++					4	4 / 0
Frec.	2^{W}	4	2	5	3	0	16^{Y}	13 / 3
[mM]							Selection ≥ 3	
	Firmness µ 10.05 (8.87 – 11.38 lb in^2) R^2 0.9606 C.V. 3.40							
27.54	++		++		+++		7	7 / 0
17.70	+++			--			5	3 / 2
-25.30		+++				++	5	5 / 0
Frec.	5	3	2	2 C	3	2	17	15 / 2
[mM]	1.285^{X}	13.24	0.123	0.052	1.196	0.043	Selection ≥ 3	
	Total soluble solids µ 13.55 (12.80 – 14.60 °Brix) R^2 0.8751 C.V. 4.61							
27.29					+++		3	3 / 0
9.61	++++						4	4 / 0
-25.29		++++					4	4 / 0
Frec.	4	4	0	0	3	0	11	11 / 0
[mM]							Selection ≥ 2	
	Titratable acidity µ 0.3363 (0.2747 – 0.3953% malic acid) R^2 0.9981 C.V.1.42							
33.24		+++	++				5	5 / 0
-14.18	++			---			5	2 / 3
-41.12	++				+++		5	5 / 0
Frec.	4	3	2	3	3	0	15	12 / 3
[mM]							Selection ≥ 3	
	Rel. Sugar Acidity µ 40.67 (32.89 – 48.05 °Brix / malic acid) R^2 0.9899 C.V. 3.77							
62.73	++				+++		5	5 / 0
22.38	+++			---			6	3 / 3
-52.04		+++					3	3 / 0
Frec.	5	3	0	3	3	0	14	11 / 3
[mM]							Selection ≥ 3	
	Juice density µ 1.82 (1.33 – 2.22 g ml^{-1}) R^2 0.9993 C.V.1.17							
38.16	++				+++		5	5 / 0
18.43			---				3	0 / 3
-38.46	--	+++					5	3 / 2
Frec.	4	3	3	0	3	0	13	8 / 5
[mM]							Selection ≥ 3	
	Percentage of juice µ 63.33 (39.25 – 80.31%) R^2 0.9555 C.V. 11.74							
56.99			++	++	++		6	6 / 0
17.84	+++	++		--			7	5 / 2
-53.91		++++					4	4 / 0
Frec.	3	6	2	4	2	0	17	15 / 2
[mM]	1.175	15.00	0.098	0.049	0.816	0.046	Selection ≥ 3	
	Total phenols µ 434.097 (292.419 – 600.484 µg gal acid gr^{-1} p.f) R^2 0.8649 C.V. 6.17							

79.35	---		++				5	2 / 3
40.43	++	--	++			++	8	6 / 2
-50.95		+++	++			++	7	7 / 0
Frec	5 L C; SA^{W}	5 L C; SA,	6 C	0	0 L C	4 L C	20	15 / 5
[mM]	0.383**^{X}	10.24**	0.130**	0.051*	1.065**	0.043**	Selection ≥ 4	
	Antioxidant capacity µ 3.261 (1.528 – 4.633 mg trolox g^{-1} p.f.) R^2 0.9512 C.V. 5.19							
151.10			++		+++		5	5 / 0
104.19	++++						4	4 / 0
-141.89		+++				++	5	5 / 0
Frec.	4	3	2	0	3	2	14	14 / 0
[mM]							Selection ≥ 3	
	Summary						Tot. prop. +/-	
Subtotal	36	34	19	17	23	8	137	
Selection	3 / 9	3 / 9	1 / 9	1 / 9	1 / 9	1 / 9	Variables 3/9	
Prop. +/-	31 / 5	32 / 2	16 / 3	4 / 13	23 / 0	8 / 0	114^{Z} / 23	
[mM]	1.285	15.00			1.196		Selec.≥ 23 (17)	

^{S}Simple mean factor levels; ^{T}Eigenvalues expressed as a percentage of the mean of the response variable; ^{U}Range in parentheses corresponds to the predicted values from the simple mean; ^{V}Each sign corresponds to multiples of 0.25 rounded to the nearest quarter; ^{W}linear regression response type L, quadratic C and factor interaction; ^{X}Optimal value of the predicted factors and probability: significant * (0. 05 ≤ Pr ≤ 0.01), highly significant ** (Pr <0.01), otherwise not significant; ^{Y}Total observed frequency for that variable, ^{Z}Total frequency for the set of variables, factors with a subtotal equal to or greater than 20% are selected while variables greater than or equal to 15% are selected

Table S6. Selection of factors, quality variables and bioactive compounds of apple, stored in a controlled atmosphere for 7 months and left on the shelf for 13 days

Eigenvalues	Factors / simple average [mM]							
	K **1.125**[S]	Ca **15.75**	Co **0.090**	Mo **0.045**	SA **0.720**	Mg **0.045**	Eigenvectors Tot. Prop. +/-	
	Colour[U] µ 66.71 (62.29 – 69.48%) R^2 0.9195 C.V. 2.69							
Frec.	2[W]	4	2	5	3	0	16[Y]	13 / 3
[mM]							Selection ≥ 3	
	Firmness µ 10.05 (8.87 – 11.38 lb in^2) R^2 0.9606 C.V. 3.40							
Frec.	5	3	2	2 C	3	2	17	15 / 2
[mM]	1.285[X]	13.24	0.123	0.052	1.196	0.043	Selection ≥ 3	
	Total soluble solids µ 13.55 (12.80 – 14.60 °Brix) R^2 0.8751 C.V. 4.61							
Frec.	4	4	0	0	3	0	11	11 / 0
[mM]							Selection ≥ 2	
	Titratable acidity µ 0.3363 (0.2747 – 0.3953% malic acid) R^2 0.9981 C.V.1.42							
Frec.	4	3	2	3	3	0	15	12 / 3
[mM]							Selection ≥ 3	
	Rel. Sugar Acidity µ 40.67 (32.89 – 48.05 °Brix / malic acid) R^2 0.9899 C.V. 3.77							
Frec.	5	3	0	3	3	0	14	11 / 3
[mM]							Selection ≥ 3	
	Juice density µ 1.82 (1.33 – 2.22 g ml^{-1}) R^2 0.9993 C.V.1.17							
Frec.	4	3	3	0	3	0	13	8 / 5
[mM]							Selection ≥ 3	
	Percentage of juice µ 63.33 (39.25 – 80.31%) R^2 0.9555 C.V. 11.74							
Frec.	3	6	2	4	2	0	17	15 / 2
[mM]	1.175	15.00	0.098	0.049	0.816	0.046	Selection ≥ 3	
	Total phenols µ 434.097 (292.419 – 600.484 µg gal acid gr^{-1} p.f) R^2 0.8649 C.V. 6.17							
Frec	5 L C; SA[W]	5 L C; SA	6 C	0	0 L C	4 L C	20	15 / 5
[mM]	0.383**[X]	10.24**	0.130**	0.051*	1.065**	0.043**	Selection ≥ 4	
	Antioxidant capacity µ 3.261 (1.528 – 4.633 mg trolox g^{-1} p.f.) R^2 0.9512 C.V. 5.19							
Frec.	4	3	2	0	3	2	14	14 / 0
[mM]							Selection ≥ 3	
	Summary						Tot. prop.+/-	
Subtotal	36	34	19	17	23	8	137	
Selection	3 / 9	3 / 9	1 / 9	1 / 9	1 / 9	1 / 9	Variables 3/ 9	
Prop. +/-	31 / 5	32 / 2	16 / 3	4 / 13	23 / 0	8 / 0	114[Z] / 23	
[mM]	1.285	15.00			1.196		Selec.≥ 23 (17)	

[S]Simple mean factor levels; [U]Range in brackets corresponds to the predicted values from the simple mean; [W]linear regression response type L, quadratic C and factor interaction; [X]Optimal value of the predicted factors and probability: significant * (0.05 ≤ Pr ≤ 0. 01), highly significant ** (Pr <0.01), otherwise not significant; [Y]Total observed frequency for that variable, [Z]Total frequency for the set of variables, those factors with a subtotal equal or greater than 20% are selected while variables greater or equal to 15% are selected