Research Article

Received: 7 August 2009 Revised: 11 November 2009 Accepted: 19 November 2009 Published online in Wiley Interscience: 12 January 2010

(www.interscience.wiley.com) DOI 10.1002/jsfa.3868

Effect of ethylene and 1-MCP treatments on strawberry fruit ripening

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Abstract

BACKGROUND: Strawberry is a soft fruit, considered as non-climacteric, being auxins the main hormones that regulate the ripening process. The role of ethylene in strawberry ripening is currently unclear and several studies have considered a revision of the possible role of this hormone.

RESULTS: Strawberry fruit were harvested at the white stage and treated with ethephon, an ethylene-releasing reagent, or 1-methylcyclopropene (1-MCP), a competitive inhibitor of ethylene action. The effects of the treatments on fruit quality parameters and on the activity of enzymes related to anthocyanin synthesis and cell wall degradation were evaluated. Some aspects of ripening were accelerated (anthocyanin accumulation, total sugar content and increment of phenylalanine ammonia-lyase (PAL; EC 4.3.1.24) and β-galactosidase (EC 3.2.1.23) activities), while others were repressed (chlorophyll levels and increment of endo-1,4-β-glucanase (EC 3.2.1.4) and β-xylosidase (EC 3.2.1.37) activities) or unchanged (reducing sugar content, pH, titratable acidity and α-L-arabinofuranosidase (EC 3.2.1.55) activity) by ethylene. 1-MCP treatment caused the opposite effect. However, its effects were more pronounced, particularly in anthocyanin accumulation, phenolics, PAL and polygalacturonase (EC 3.2.1.15 and EC 3.2.1.67) activities.

CONCLUSION: These observations probably indicate that strawberry produces low levels of ethylene that are sufficient to regulate some ripening aspects.

Keywords: strawberry; ethylene; 1-methylcyclopropene; ripening

INTRODUCTION

Strawberry (*Fragaria* × *ananassa*, Duch.) is a highly perishable non-climacteric fruit, which suffers extensive changes in colour, flavour, aroma and texture during ripening.[1] In spite of the economic importance of this fruit, little is known about the mechanisms that regulate its ripening. Although a clear role was established for auxins as negative regulators of strawberry receptacle ripening, no growth regulator seems to play a positive role analogous to that played by ethylene in the ripening of climacteric fruits.[2] It has been reported that treatments with exogenous ethylene could accelerate ripening of non-climacteric fruits.[3,4] In the case of strawberry, results are contradictory and no clear relationship between ethylene and strawberry ripening has yet been established. Treatment with ethylene inhibitors such as silver ions, norbornadiene and aminoethoxyvinylglycine in 'Brighton' cultivar did not affect fruit ripening.[1] However, strawberries of 'G-3' and 'G-4' cultivars exposed to ethylene developed a more intense red colour and softened more quickly than those stored in ethylene-free air.[5] Jiang *et al*. demonstrated that 1-methylcyclopropene (1-MCP), a competitive inhibitor of ethylene action, delays changes in strawberry fruit firmness and colour.[6]

Effects of ethylene and 1-MCP treatments on strawberry fruit quality have been analysed at commercial ripening stage.[7] However, most changes associated with ripening start at the white stage, when the fruit has reached almost its final size and the accumulation of anthocyanins has begun.[8] Moreover, it has been reported that strawberry fruit would be more responsive to treatment with exogenous ethylene at the white stage than older fruits.[9] For these reasons, we investigated the role of ethylene in strawberry fruit ripening, applying ethephon (an ethylene-generating compound) or 1-MCP (an ethylene perception inhibitor) on white fruit of 'Toyonoka' cultivar and analysed their effects on fruit quality parameters such as sugars, phenols, acidity and pigments. Also, we analysed the activity of enzymes related to anthocyanin metabolism (phenylalanine ammonia-lyase (PAL)) and pectin and hemicellulose degradation (endo-1,4-β-glucanase (EGase), polygalacturonase (PG), β-galactosidase (β-Gal), β-xylosidase (β-Xyl) and α-L-arabinofuranosidase (α-Ara)).

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EXPERIMENTAL

Plant material

Strawberry (*Fragaria* × *ananassa* Duch.) fruit were obtained from local producers (La Plata, Buenos Aires Province, Argentina). Fruit from 'Toyonoka' (low-firmness) cultivar were harvested at white stage (W), since at this point its final size is almost reached and anthocyanin accumulation has just begun. The peduncle of each fruit was cut at 30 mm from the receptacle base, and fruit were washed, drained, classified according to shape and size, and used in plant growth regulators assays.

Hormone treatments

Ethephon was applied according to usual practices in fruit postharvest treatments.[10] Whole fruit were submerged for 5 min in a solution of 2 mmol L^{-1} ethephon (2-chloroethylphosphonic acid, an ethylene-releasing agent) in 0.2 mL L^{-1} Tween 20 and 10 mL L^{-1} ethanol (both used as surfactants), prepared immediately before use. Controls were submerged for the same time in 0.2 mL L^{-1} Tween 20 and 10 mL L^{-1} ethanol. Immersion treatments were applied at 22 °C, and then fruit were vertically placed on microcentrifuge tubes with their peduncles in contact with distilled water.

In the case of 1-MCP treatment, fruit with peduncles in contact with distilled water were treated with this inhibitor (cc = 1 µL L^{-1}) in a hermetic container for 10 h, while the control were kept under the same conditions without 1-MCP.

Treated and control fruit were placed in a growth chamber at 22 °C for 48 h. After storage, the calyx and peduncle were removed and fruit were cut apart, frozen in liquid nitrogen and stored at −80 °C until use.

Anthocyanins

Frozen fruit (10 g) were ground with a mortar and pestle in the presence of liquid nitrogen. Approximately 0.3 g of the resultant powder was poured into 3 mL of HCl–methanol 10 mL L^{-1} and held at 0 °C for 10 min. The slurry was centrifuged at 1500 × *g* at 4 °C for 10 min, the supernatant was recovered and its optical density (OD) at 515 nm was measured. The amount of anthocyanins was expressed as micromoles of pelargonidine-3-glucoside per kilogram of fruit, using $E_{molar} = 3.6 \times 10^6$ L mol^{-1} m^{-1}.[11]

Chlorophylls

The methodology to measure chlorophyll content was adapted from Inskeep and Bloom.[12] Frozen fruit were ground with a mortar and pestle in the presence of liquid nitrogen, and 0.5 g of the powder was suspended with 3 mL of dimethylformamide (DMF). The mixture was centrifuged at 10 000 × *g* for 10 min at 4 °C, the supernatant was recovered and OD was measured at 647 and 664.5 nm. Results were expressed as grams of chlorophylls per kilogram of fresh fruit.

Reducing and total sugar content

Frozen fruit were ground with a mortar and pestle in the presence of liquid nitrogen, and 0.4 g of the powder was suspended with 6 mL of ethanol. The mixture was centrifuged at 9000 × *g* for 10 min at 4 °C, and 1 mL of the supernatant was brought to 50 mL with water. The content of reducing sugar was determined spectrophotometrically at 540 nm using a modification of the Somogyi–Nelson method.[13] For total sugar determination, 0.1 mL of extract was mixed with 1 mL of 2 g L^{-1} anthrone in 0.72 L L^{-1} H_2SO_4. The mixture was incubated at 100 °C for 12 min, cooled in a water bath at room temperature, and the sugar content was measured spectrophotometrically at 625 nm. Results were expressed as grams of glucose per kilogram of fresh fruit.

Total phenolic compounds

Frozen fruit were ground with a mortar and pestle in the presence of liquid nitrogen, 1 g of the powder was suspended with 6 mL of ethanol and the mixture was centrifuged at 9000 × *g* for 10 min at 4 °C. One millilitre of the resultant supernatant was brought to 5 mL with water and this solution was used to determine total phenolic compounds according to Zieslin and Ben-Zaken.[14] Results were expressed as grams of phenol per kilogram of fruit.

pH and titratable acidity

Frozen fruit were ground with a mortar and pestle in the presence of liquid nitrogen, and 10 g of the powder was suspended with water up to a volume of 100 mL. The pH of the homogenate was measured and the acidity was determined by titration with 0.01 mol L^{-1} NaOH up to pH 8.1, according to AOAC.[15] Titratable acidity was expressed as milliequivalents of H^+ kg^{-1} of fresh fruit. Two independent samples per condition were analysed and each sample was titrated by duplicate.

Phenylalanine ammonia-lyase activity

About 5 g of fruit were homogenized in an Omnimixer at 4 °C with 4 volumes of buffer of the following composition: 0.1 mol L^{-1} $Na_2B_4O_7.10\ H_2O$, 0.05 mol L^{-1} 2-mercaptoethanol, 0.02 mol L^{-1} ethylenediaminetetraacetic acid (EDTA), 30 g L^{-1} polyvinylpolypyrrolidone (PVPP), pH 8.8. The mixture was left for 1 h at 4 °C under magnetic stirring and then centrifuged at 10 000 × *g* for 20 min at 4 °C. The enzymatic activity was measured in the supernatant by the method described by Zucker.[16] Results were expressed as the change in optical density (ΔOD) in a second per kilogram of fresh fruit.

Polygalacturonase and endo-1,4-β-glucanase

Frozen strawberries (5 g) were homogenized in an Omnimixer with 15 mL of the following buffer: 0.05 mol L^{-1} sodium acetate/acetic acid, 10 g L^{-1} PVPP, pH 6.0. The mixture was centrifuged at 12 000 × *g* for 30 min and the supernatant was discarded. The pellet was washed twice with 15 mL of buffer A (0.05 mol L^{-1} sodium acetate/acetic acid pH 6.0). Then, the sample was centrifuged at 12 000 × *g* for 30 min, the supernatant was discarded and the pellet was suspended with 15 mL of buffer A containing 1 mol L^{-1} NaCl. The mixture was stirred for 2 h and then centrifuged at 12 000 × g for 30 min. The supernatant was saved for assaying both activities.

In the case of EGase, the reaction mixture contained 0.05 mol L^{-1} sodium acetate/acetic acid pH 6.0, 1 mol L^{-1} NaCl, 5 g L^{-1} carboxymethyl cellulose and 1.5 mL of enzymatic extract in a total volume of 2 mL. The mixture was incubated at 37 °C and aliquots of 150 µL were taken at different times. Sugars were determined by the dinitrosalicylic acid (DNS) method according to Miller.[17]

Polygalacturonase (PG) activity was measured as described previously.[18] Results were expressed as nanomoles of galacturonic acid released per second per kilogram of fruit.

β-Xylosidase and β- galactosidase activity

Frozen strawberries (5 g) were homogenized in an Omnimixer with 15 mL of buffer B (0.05 mol L^{-1} sodium acetate/acetic acid, 1 mol L^{-1} NaCl, 10 g L^{-1} PVPP, pH 6.0). The mixture was left under stirring for 2 h and then centrifuged at 10 000 × *g* for 10 min. The supernatant was used to determine both enzyme activities.

β-Xyl activity was measured as described previously,[19] while β-Gal activity was assayed according to Trainotti *et al*.[9]

In both cases, results were expressed as nmol of *p*-nitrophenol released per second per kilogram of fruit.

α-L-Arabinofuranosidase activity

α-Ara activity was measured as described by Rosli *et al*.[20] Results were expressed as nanomoles of 4-nitrophenol released per second per kilogram of fruit.

Experimental design and statistical analysis

Each hormone treatment was performed with 15 fruit, while another 15 fruit were maintained untreated as controls. The whole experiment was repeated three times.

For anthocyanins, chlorophylls, reducing and total sugar content and phenolics, measurements were performed in triplicate for each condition analysed. For enzymatic assays, two independent extracts were prepared for each condition analysed, and the activity for each extract was measured twice.

Data for enzymatic activities, anthocyanins, chlorophylls, phenolic compounds, sugar content, pH and titratable acidity were analysed by analysis of variance, and the means were compared by the LSD test at a significance level of 0.05.

RESULTS AND DISCUSSION

Anthocyanins, chlorophylls and phenylalanine ammonia-lyase activity

In strawberry, an increase in PAL activity is necessary for the accumulation of anthocyanins during ripening.[1] We detected an increase in anthocyanin content and PAL activity in all fruit after 48 h of incubation at 22 °C, in comparison with the values found in fruit at the initial white stage (Fig. 1(A) and (B)). Fruit treated with ethephon accumulated more anthocyanin than the corresponding controls, while the opposite was observed in fruit treated with 1-MCP (Fig. 1(A)). In accordance with this, PAL activity increased significantly in ethephon-treated fruit with regard to controls, while in fruit treated with 1-MCP the activity was three times lower than controls (Fig. 1(B)). In grape berries, other fruit considered as non-climacteric, it has been reported that treatments with exogenous ethylene were also able to stimulate anthocyanin accumulation and the expression of genes related to anthocyanin biosynthesis.[4]

In addition to the increase in anthocyanin content, strawberry fruit ripening is accompanied by a decrease in chlorophyll levels.[1] We detected a degradation of these pigments in fruit incubated for 48 h at 22 °C. Moreover, the decrease in total chlorophyll levels was more pronounced in fruit treated with ethephon with regard to controls, and the opposite situation was observed in 1-MCP-treated fruit (Fig. 1(C)).

Our results reinforce the idea that ethylene could play a role in ripening regulation in a non-climacteric fruit such as strawberry. In particular, ethylene could stimulate anthocyanin accumulation and degradation of chlorophylls in this fruit.

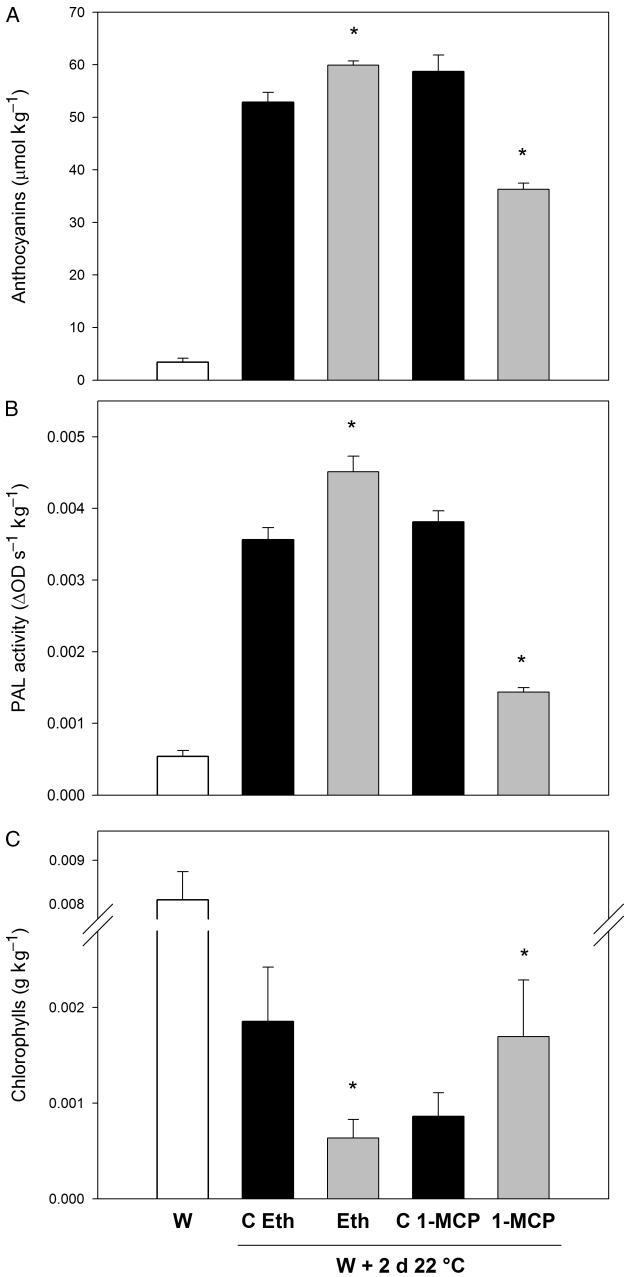

Figure 1. Effect of ethephon and 1-MCP treatments on anthocyanin content (A), PAL activity (B) and chlorophyll levels (C). White fruit (W) was treated with ethephon (2 mmol L^{-1}) or 1-MCP (1 µg L^{-1}) and incubated for 2 days at 22 °C. Bars indicate standard deviations. Asterisks show significant differences at $P < 0.05$ with the corresponding control. C Eth, control of ethephon treatment; Eth, ethephon treatment; C 1-MCP, control of 1-MCP treatment; 1-MCP, 1-MCP treatment.

Sugars, phenolic compounds, pH and titratable acidity

Previous studies have shown that strawberry fruit did not accumulate appreciable amount of sugars after harvest.[21] Similarly, we did not detect an increase in total sugar levels in control fruit after 48 h of incubation at 22 °C with regard to initial levels. Nonetheless, fruit treated with ethephon accumulated higher amounts of sugar than controls, while 1-MCP-treated fruit had lower levels of total sugars than controls after incubation (Fig. 2(A)). A similar behaviour was described in grape berries: fruit treated with 1-MCP *in planta* accumulated less sucrose than

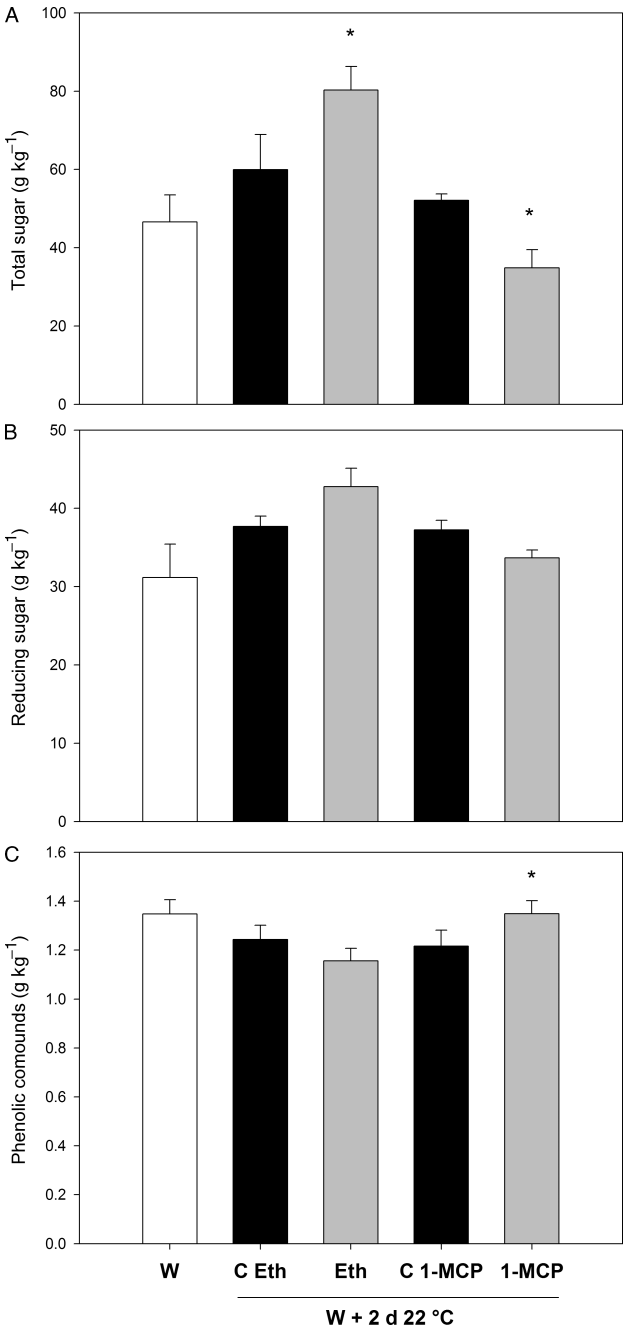

Figure 2. Effect of ethephon and 1-MCP treatments on total (A) and reducing (B) sugar content, and on total phenolic compounds (C). White fruit (W) was treated with ethephon (2 mmol L^{-1}) or 1-MCP (1 µg L^{-1}) and incubated for 2 days at 22 °C. Bars indicate standard deviations. Asterisks show significant differences at $P < 0.05$ with the corresponding control. C Eth, control of ethephon treatment; Eth, ethephon treatment; C 1-MCP, control of 1-MCP treatment; 1-MCP, 1-MCP treatment.

control fruit, suggesting a role of ethylene in sugar accumulation.[22] We also measured reducing sugar content and, even when similar tendencies were observed, significant differences between treated and control fruit were not detected in any case (Fig. 2(B)).

Our results indicate that ethylene could be upregulating some mechanism related to sugar accumulation during postharvest. This statement is supported by results obtained with 1-MCP treatments (Fig. 2(A)).

Phenolic compound content decreases continuously during strawberry fruit ripening, and the most significant drop is observed from small green (SG) to white stage.[23] In fruit treated with ethephon and corresponding controls, a decrease in levels of total phenolic compounds was detected after 2 days of incubation (Fig. 2(C)). However, no difference between treated and control fruit was found. The set of fruit treated with 1-MCP showed the same levels of phenolic compounds as the initial white fruit, while the corresponding controls showed a decline in total phenolic compounds. Then, treatment with 1-MCP reduced significantly the decrease of phenolic compounds during fruit incubation.

PAL is a key enzyme in the biosynthesis of phenolics.[24] In olive drupes, a clear correlation has been observed between PAL activity and phenolic content.[25] Conversely, in the case of cherimoya, an increase in PAL activity did not have a corresponding impact on the total phenolic content during fruit ripening.[26] In the case of strawberry, we observed an increase in PAL activity and a decrease in phenolics during ripening, indicating that there is not a direct correlation between them. However, this observation seems to be cultivar-dependent. In 'Everest' cultivar, a direct correlation between PAL activity and phenolic compounds was reported.[6] Altogether, these results indicate that it is not possible to establish a clear relationship between changes in phenolics and PAL enzymatic activity during fruit ripening.

The pH of strawberry juice was not significantly modified under any of the conditions analysed, and no difference in titratable acidity was found either (Table 1).

Cell wall hydrolases

Strawberries accumulate sugars during ripening by importing them from the leaves. As starch is degraded rapidly as fruit ripen, and the source of sugar is lost after harvest, fruit would be depleted of sugars during postharvest.[27] In this sense, the only possible source of simple sugars should be the degradation of cell wall components. Moreover, in *Arabidopsis*, it was shown that glycosyl hydrolases of cell wall are induced by sugar starvation.[28] Results described above indicate that ethylene plays a role in sugar accumulation. If these sugars come from cell wall polymers, then ethylene might influence changes in cell wall hydrolases during postharvest.

Polygalacturonase, β-galactosidase and α-L-arabinofuranosidase

In strawberry, the depolymerization and solubilization of pectins increase during ripening and contribute to fruit softening. In 'Toyonoka' cultivar, pectin depolymerization was detected during ripening, particularly in the covalently bound fraction.[29] A number of hydrolases, mainly polygalacturonases, have been implicated in this process. Endo- and exo-polygalacturonases catalyze the cleavage of homogalacturonan backbones. It has been suggested that PG could be one of the enzymes that determine the different softening rate detected among cultivars of strawberry fruit.[18] Moreover, the accumulation of two PG mRNAs (*FaPG1* and *T-PG*) and FaPG1 synthesis increased considerably in response to ethylene in 'Camarosa' fruit, while the opposite result was observed with 1-MCP treatment.[30] In the present study, total PG activity slightly increased after 48 h incubation in both ethephon and 1-MCP controls (Fig. 3(A)). The increment of total PG activity was not significantly modified by ethylene treatment, but the application of 1-MCP maintained PG activity at levels similar to initials, suggesting that ethylene might play a role in the upregulation of polygalacturonase expression in strawberry fruit.

Table 1. Effect of ethephon (2 mmol L^{-1}) and 1-MCP (1 µL L^{-1}) on pH and titratable acidity of strawberry fruit

	W (white) fruit + 2 days 22 °C				
	W fruit	C Eth	Eth	C 1-MCP	1-MCP
PH	3.64 ± 0.02	3.60 ± 0.02	3.57 ± 0.01	3.54 ± 0.02	3.52 ± 0.00
Titratable acidity (H^+ meq kg^{-1})	198.02 ± 0.00	225.30 ± 6.00	231.03 ± 2.99	220.43 ± 14.22	220.18 ± 9.17

C Eth: control of ethephon treatment; Eth: ethephon treatment; C 1-MCP: control of 1-MCP treatment; 1-MCP: 1-MCP treatment.

Solubilization of pectic polymers in strawberry fruit has been associated with hydrolysis of residues present in their side chains, mainly arabinose and galactose.[29] β-Galactosidases remove non-reducing terminal galactosyl residues from side chains of pectin polysaccharides. In strawberry, β-Gal activity could be detected from the large green (LG) stage and increased many-fold thereafter.[31] We detected an increase in total β-Gal activity after 2 days of incubation in all the sets of fruit compared to the initial white stage (Fig. 3(B)). It is worth noting that a significant increment in β-Gal activity was observed in fruit treated with ethephon, while those that were treated with 1-MCP showed a significant reduction in relation to the controls (Fig. 3(B)). These results suggest a positive regulation of β-Gal activity by ethylene. The effect of ethylene on β-Gal gene expression has been studied in strawberry fruit.[31] The authors mentioned that ethylene treatment did not seem to affect the expression of different β-Gal genes (*FaβGal1*, *2* and *3)* in SG fruit. However, in W fruit, ethylene appeared to have a negative effect on *FaβGal1* expression. As the authors did not analyse the effect of the hormone on the expression of *FaβGal2* and *3* genes or on total β-Gal activity in W fruit, other enzymes different from Faβ Gal1 could be responsible for the increase and decrease that we observed in total enzymatic activity after ethephon and 1-MCP treatments, respectively.

α-Ara catalyses the hydrolysis of terminal α-L-arabinofuranosyl residues from various pectic and hemicellulosic polysaccharides.[32] In strawberry, α-Ara activity is undetectable in SG stage and increases from W to ripe fruit, this increment being higher in soft fruit such as those from 'Toyonoka' cultivar.[20] In accordance with that report, we detected an increase in α-Ara enzyme activity after 48 h of incubation at 22 °C. However, neither treatment with ethephon nor treatment with 1-MCP induced changes in α-Ara activity compared to controls (Fig. 3(C)), suggesting that ethylene might not regulate α-Ara activity. A similar behaviour was observed for α-Af I and II isoform activities in tomato, where ethylene treatment did not affect the activity of these isoforms, although it increased the activity of α-Af III.[32]

Endo-1,4-β-glucanase and β-xylosidase

Hemicelluloses, which mainly include xyloglucans and xylans, show a slight depolymerization during strawberry fruit ripening.[29] EGases are enzymes active against β-(1,4)-glucan links. Although little is known about their real substrate *in vivo*, xyloglucans and non-crystalline regions of cellulose have been proposed as their natural substrates in the cell wall. EGase activity has been reported in strawberry fruit, particularly in the overripe stage.[33] Total endo-1,4-β-glucanase enzyme activity increased in both ethephon and 1-MCP controls during storage (Fig. 4(A)). Treatment with ethephon caused complete inhibition of the increment of EGase activity, while an opposite situation was observed with 1-MCP treatment, where EGase activity increased. These results suggest a negative influence of ethylene on EGase activity. To our knowledge, this is the first report about the ethylene regulation of EGase enzymatic activity in strawberry fruit. In the case of gene transcription, Balogh *et al*. identified a putative endo-1,3-1,4-β-D-glucanase that is upregulated by 1-MCP in green strawberry fruit, suggesting a downregulation of gene expression by ethylene.[34]

In strawberry fruit, xylose subunits represent nearly 30% of hemicellulose composition and are also present in small amounts in pectins.[35] β-Xylosidases are enzymes involved in the degradation of xylans, liberating xylose. The participation of β-xylosidases in cell wall metabolism has been proposed since the downregulation of a β-xylosidase gene altered cell wall composition and plant development in *Arabidopsis thaliana*.[36] It was shown that β-xylosidase activity and the expression of a related gene (*FaXyl1*) are higher in a softer cultivar ('Toyonoka') compared to a firmer one ('Camarosa'). In 'Toyonoka', β-xylosidase activity increases from LG to W stage and then decreases in 50% red fruit (R). Subsequently, activity increases again in ripe fruit.[19] In the present work, we detected a rise in enzymatic activity after 2 days of incubation as regards white fruit (Fig. 4(B)). The effect of ethephon and 1-MCP treatments was similar to that observed for EGase activity. Ethephon-treated fruit showed a decrement in β-Xyl activity, reaching values significantly lower than those of controls. Conversely, fruit treated with the inhibitor of ethylene perception showed a slight increase in the enzyme activity in relation to the respective controls. Both experiments indicate that, at least in cultivar 'Toyonoka', β-xylosidase activity would be repressed by ethylene.

Finally, the increment in EGase and Xyl activities observed during strawberry fruit ripening would be regulated by other factors besides ethylene.

CONCLUSIONS

Strawberry has long been considered a non-climacteric fruit owing to its scarce ethylene production during ripening and its slight or null response to exogenous ethylene. However, the possible role of ethylene in non-climacteric fruits has been reviewed in the recent and it has been shown that this gaseous hormone could influence particular aspects of some non-climacteric fruit.[3,4] In the case of strawberry, experiments done up until now arrived at non-concordant conclusions. Several reasons could explain why results are contradictory. First, the stage of ripening in which fruit react to an ethylene treatment may be important in strawberry. Recently, three cDNAs from strawberry encoding different ethylene receptors have been cloned and characterized.[9] One of them (*FaEtr2*) has a higher expression at the white ripening stage. Moreover, the authors reported that two of these receptors were more responsive to ethylene in white fruit than in red ones. In this work, we found that several aspects related to strawberry ripening were modulated by ethylene or 1-MCP.

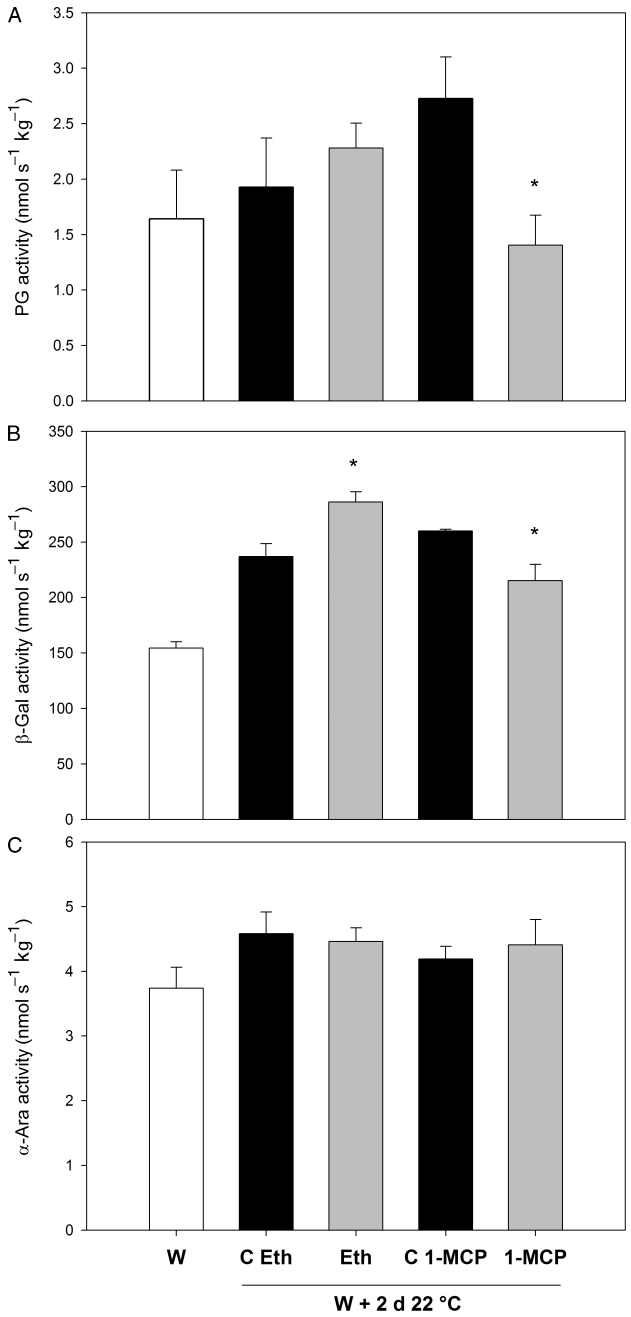

Figure 3. Effect of ethephon and 1-MCP treatments on PG (A), β-Gal (B) and α-Ara (C) activities. White fruit (W) was treated with ethephon (2 mmol L^{-1}) or 1-MCP (1 µg L^{-1}) and incubated for 2 days at 22 °C. Bars indicate standard deviations. Asterisks show significant differences at $P < 0.05$ with the corresponding control. C Eth, control of ethephon treatment; Eth, ethephon treatment; C 1-MCP, control of 1-MCP treatment; 1-MCP, 1-MCP treatment.

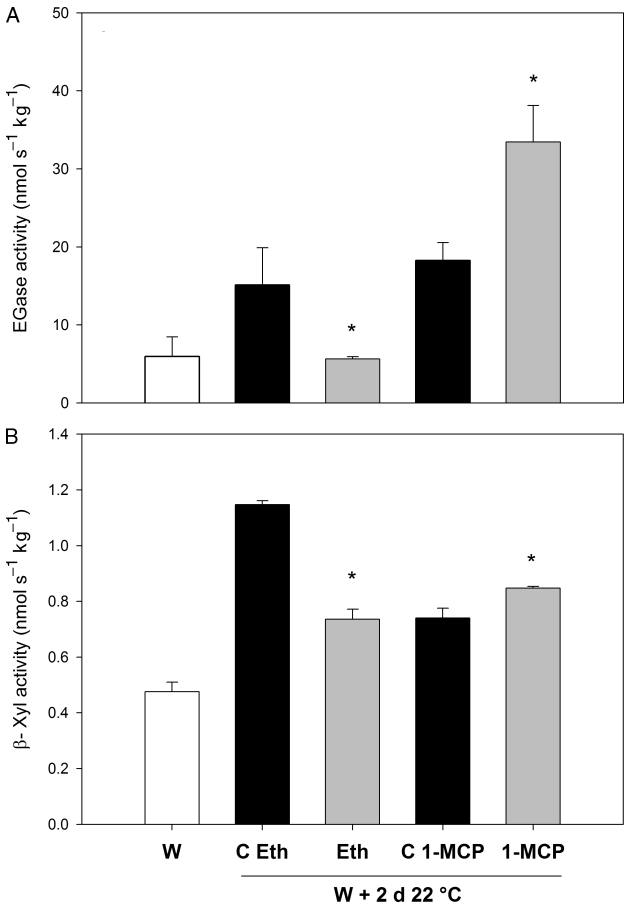

Figure 4. Effect of ethephon and 1-MCP treatments on EGase (A) and β-Xyl (B) activities. White fruit (W) was treated with ethephon (2 mmol L^{-1}) or 1-MCP (1 µg L^{-1}) and incubated for 2 days at 22 °C. Bars indicate standard deviations. Asterisks show significant differences at $P < 0.05$ with the corresponding control. C Eth, control of ethephon treatment; Eth, ethephon treatment; C 1-MCP, control of 1-MCP treatment; 1-MCP, 1-MCP treatment.

Second, different strawberry cultivars can show differences in their metabolism. It has been shown that cell wall metabolism can greatly vary among cultivars.[18,19,29] In this context, differences in the effects caused by ethylene treatments could be due to differences in responses among cultivars. Third, some aspects of ripening could be modulated by ethylene, while others might remain completely insensitive. In this work, we observed that anthocyanin amount, total sugar content, PAL and β-Gal activities were upregulated, while chlorophyll levels, EGase and β-Xyl activities were downregulated by ethylene. Moreover, reducing sugar content, pH, titratable acidity and α-Ara activity were not affected by ethylene. Fourth, although endogenous ethylene production is scarce, it may have some effects in a particular ripening feature. It could also occur that these low ethylene levels are enough to influence the ripening process in this fruit, and that an additional application does not induce a further response. In this sense, application of inhibitors of ethylene action would cause the opposite effect to ethylene applications but to a greater extent. In this work, we found that inhibition of anthocyanin accumulation and PAL activity by 1-MCP was greater than activation by ethylene. Moreover, the application of ethylene did not modify the amount of phenolic compounds and PG activity, while treatment with 1-MCP clearly did. The growing evidence given by different authors suggests that the role of ethylene in ripening of non-climacteric fruit, particularly in strawberry, should be reconsidered.

ACKNOWLEDGEMENTS

This work has been supported by grants from CONICET (PIP 5488) and ANPCYT (PICT 11040).

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