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THE INFLUENCE OF STORAGE CONDITIONS AND 1-MCP TREATMENT ON ETHYLENE EVOLUTION AND FRUIT QUALITY IN 'GALA' APPLES

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Summary

The effect of storage conditions and 1-MCP treatment on ethylene production and quality of 'Gala' apples stored in normal and controlled atmosphere conditions was evaluated during two successive seasons, 2001/2002 and 2002/2003. Apples were harvested in a commercial stage and cooled down to a temperature of 1°C. After that there were treated with 1-MCP in a concentration of 0.625 $\mu l \cdot L^{-1}$ for 24 hours. Treated and untreated (control) apples were stored for 120 and 180 days in the cold storage rooms at 2±0.5°C under normal atmosphere (NA) conditions or controlled atmosphere (CA) with 2% O_2 and 2% CO_2. Directly after storage and after shelf life (additionally 7 days at 18°C) internal ethylene concentration (IEC) and activity of ACC oxidase were determined. The following quality parameters were recorded as well: flesh firmness (FF), total soluble solids (TSS), and titratable acidity (TA).

Application of 1-MCP after harvest strongly inhibited ACC oxidase in apples following storage in air or controlled atmosphere for 120 and 180 days. This inhibition was also noted after 7 days of shelf life. There was no reduction in ACC oxidase activity of CA stored apples in comparison to NA stored, but the IEC directly after storage was lower for fruits stored in CA conditions than for fruits stored in NA. Internal ethylene concentration was significantly lower for the 1-MCP treated fruits, both directly after storage in NA or CA and after shelf life. The application of 1-MCP delayed softening and loss of titratable acidity in 'Gala' apples stored in NA and CA conditions. There was no clear effect of 1-MCP treatment on total soluble solids content in stored 'Gala' apples

key words: ACC oxidase, apples, ethylene, 1-methylcyclopropene, fruit quality, storage

INTRODUCTION

The plant hormone ethylene plays an important role in the ripening process of climacteric fruits such as apples (Yang & Hoffman 1984). Application of

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ethylene to pre-climacteric fruits initiates and hastens the changes associated with fruit ripening and senescence. In pre-climacteric fruits ethylene production is very low, but increases rapidly at the onset of ripening. Ethylene is biosynthesized in higher plants through the following pathway: methionine → S-adenosylmethionine (SAM) → 1-aminocyclopropane-1-carboxylic acid (ACC) → ethylene (Adams & Yang 1979). The final step, the oxidation of ACC to ethylene, is catalyzed by the enzyme ACC oxidase (Fernandez-Maculet & Yang 1992, Ververidis & John 1991). In fruit storage, main techniques used to minimize the effects of ethylene and improve storage quality include reduced temperature and controlled atmosphere conditions. Optimal CA conditions lower respiration and ethylene production rates, reduce ethylene action, delay ripening and senescence. Also 1-methylcyclopropene (1-MCP), a synthetic cyclopropene, has been found to inhibit the action of ethylene in various plant tissues (Sisler & Serek 1997). 1-MCP has been shown to delay ripening of climacteric fruits including banana (Jiang *et al.* 1999), avocado (Jeong *et al*. 2003), plums (Abdi *et al*. 1998, Menniti *et al*. 2004), apricots (Dong *et al*. 2002), peaches and nectarines (Fan *et al*. 2002, Liguori *et al*. 2004), pears (Lafer 2005, Trinchero *et al*. 2004), and apples (Fan *et al*. 1999, Pre-Aymard *et al*. 2003, Watkins *et al*. 2000). For apples, treatment with 1-MCP delays the onset of the rise in ethylene production and respiration, slows the loss of firmness, titratable acidity, and green peel colour, can inhibit aroma production and reduce the incidence of the storage disorders and decay (Fan *et al*. 1999, Lafer 2003, Saftner *et al*. 2003, Watkins *et al*. 2000, Xuan & Streif 2005, Zanella 2003).

The objective of the present study was to examine the effects of 1-MCP treatment on ethylene production and quality of 'Gala' apples stored in normal and controlled atmosphere conditions.

MATERIALS AND METHODS

Fruits of cv. 'Gala' were obtained from a commercial orchard located in Dąbrowice, near Skierniewice (central Poland). Trials were conducted for two successive storage seasons, 2001/2002 and 2002/2003. Apples were picked at a commercial maturity stage based on internal ethylene concentration (0.301 $\mu l \cdot L^{-1}$ in 2001, 0.768 $\mu l \cdot L^{-1}$ in 2002), starch index (6-7 in 2001, 4-5 in 2002) and flesh firmness (82.1 N in 2001, 80.6 N in 2002), and the harvest dates were: 28th September in 2001 and 12th September in 2002. Harvested fruits were cooled down for at least 24 hours to a temperature of 1°C. After that fruits were treated with 1-MCP in a concentration of 0.625 $\mu l \cdot L^{-1}$ for 24 hours. Treated and untreated (control) apples were stored for 120 and 180 days in the cold storage rooms at 2±0.5°C under normal atmosphere (NA) conditions or controlled atmosphere (CA) with 2% O_2 and 2% CO_2. On removal from storage the fruits were placed for 7 days at 18°C to simulate shelf life. Directly after storage and after shelf life the internal ethylene concentration (IEC) and activity of ACC oxidase were determined. The following quality parameters were also recorded: flesh firmness (FF), total soluble solids (TSS), and titratable acidity (TA).

For internal ethylene concentration 1mL gas samples were taken from the apple core and injected into a HP 5890 II gas chromatograph equipped with alumina packed glass column. For assay of *in vivo* ACC oxidase activity, apple fruit discs (ca. 0.25 g) were first incubated for 1 hour at 30°C in 10-ml vials with 0.5 ml reaction mixture containing 0.1 M Tricine (pH 7.5), 30 mM ascorbic acid, 0.1 mM $FeSO_4$, 30 mM $NaHCO_3$ and 10 mM ACC. The vials were then sealed and the ethylene produced during the following 1 hour was measured. The ACC oxidase activity was expressed as nl of ethylene produced per 1 gram fresh weight per 1 hour. The measurement of IEC and ACC oxidase activity were done on a sample of ten fruits.

Flesh firmness was measured on two opposite sides of each fruit (after skin removing) using an EPT-1R (Kelowna, Canada) pressure tester equipped with a standard Magness-Taylor plunger of 11.1 mm. TSS and TA were determined in freshly prepared juice. The percentage of total soluble solids was measured using a digital electronic refractometer type PR 101 (Atago, Japan). Titratable acidity was quantified by titration of juice (automatic titrator DL 21, Mettler Toledo, Swiss) with 0.1 M NaOH to a pH 8.1 end point and was expressed in percentage of malic acid. Three replicates with ten fruits were used for each quality determination.

Data were statistically elaborated using analysis of variance, followed by means separation using Duncan's multiple range t-test at $P \leq 0.05$.

RESULTS AND DISCUSSION

The 1-methylocyclopropen (1-MCP) application after harvest markedly affected the internal ethylene concentration in 'Gala' apples after 120 and 180 days of storage (Table 1). IEC was significantly lower for the 1-MCP treated fruits, both directly after storage and after shelf life than for untreated ones. 1-MCP, an inhibitor of ethylene perception, binds irreversibly to the ethylene-binding protein (Sisler & Serek 1997), which results in inhibition of ethylene production in climacteric fruits (Dong *et al*. 2002, Pre-Aymard *et al*. 2003, Tian *et al*. 2002, Trinchero *et al*. 2004, Xuan & Streif 2005). The storage conditions affected the ethylene concentration in apples as well. Lower IEC was observed in apples stored under CA than NA conditions. The inhibition of ethylene production in apples by controlled atmosphere application has also been demonstrated (DeEll *et al*. 2005, Gorny & Kader 1996, Xuan & Streif 2005). Data presented in Table 1 showed that directly after storage IEC was lower for untreated fruits stored in CA conditions than for fruits stored in NA, but after shelf life the differences were not significantly proved, excluding apples stored for 120 days in season 2002/2003. However, the effect of 1-MCP was stronger than effect of controlled atmosphere. The most effective combination was the storage of 1-MCP treated apples under CA conditions. In both seasons the lowest concentration of ethylene was observed for those fruit, both after 120 and 180 days of storage and also after shelf life (except season 2002/2003 after 120 days of storage).

Table 1. Internal ethylene concentration (IEC) in 1-MCP treated and untreated apples after storage in NA and CA conditions and after shelf life

Length of storage (days)	Storage conditions	Treatment	Internal ethylene ($\mu l \cdot L^{-1}$)	
			After storage	After shelf life
Season 2001/2002				
120	NA	Untreated	66.213 d	184.249 c
		MCP	0.903 b	1.424 b
	CA	Untreated	26.093 c	115.587 c
		MCP	0.325 a	0.408 a
180	NA	Untreated	56.790 d	39.309 c
		MCP	4.282 b	4.095 b
	CA	Untreated	9.208 c	48.749 c
		MCP	0.180 a	0.598 a
Season 2002/2003				
120	NA	Untreated	51.695 c	117.057 c
		MCP	0.380 a	0.607 a
	CA	Untreated	9.003 b	26.164 b
		MCP	0.268 a	0.461 a
180	NA	Untreated	102.496 d	74.223 c
		MCP	1.240 b	2.390 b
	CA	Untreated	16.818 c	37.635 c
		MCP	0.333 a	0.571 a

Note: Means followed by the same letter do not differ significantly at $P \leq 0.05$ in Duncan's multiple range test (separately for each term of analysis)

The influence of 1-MCP and storage conditions on activity of ACC oxidase in apples after storage is presented in Table 2. 1-MCP treatment strongly inhibited ACC oxidase activity in fruits stored in air or controlled atmosphere for 120 and 180 days. This inhibition was also noted after 7 days of shelf life. Similar effect of 1-MCP on inhibition of ACC oxidase activity in vitro have been reported for 'Braeburn' apples (Tian *et al*. 2002). This corresponds well with results of Lelievre *et al*. (1997b), who observed that accumulation of ACC oxidase transcript was strongly reduced in 1-MCP treated pear fruits. The effect of controlled atmosphere on suppression of ACC oxidase in apples was also reported (Gorny & Kader 1996). Although controlled atmosphere strongly inhibited ethylene production (Table 1), the influence of CA storage on ACC oxidase activity was rather opposite and not completely evident (Table 2). The activity of the enzyme in untreated fruits stored in CA conditions was higher than in apples stored in NA, but the differences were not always significantly important. That tendency was also observed after 7 days of shelf life. Directly after storage the ACC oxidase activity in treated fruits was higher when apples were stored in CA conditions (season 2002/2003), but opposite effect was observed for fruits after 180 days of storage in the first year of experiment. After shelf life following 180 days of storage the activity of enzyme was the lowest for 1-MCP treated fruits in CA conditions. The relationship was no observed

after shelf life followed 120 days of storage. As reported by Ye & Dilley (1992), the accumulation of ethylene in the internal atmosphere of apple is associated with increase of ACC oxidase activity and formation of ACC, and the levels of both activities are cultivar dependent. Fernandez-Maculet & Yang (1992) demonstrated, that preclimacteric apple fruits treated with exogenous ethylene showed a marked increase in ACC oxidase activity in vitro and in vivo. In our experiment, the inhibition of ethylene production in CA stored apples was weaker than in 1-MCP treated fruits (Table 1) and therefore not enough for reducing the activity of ACC oxidase.

Table 2. Activity of ACC oxidase in 1-MCP treated and untreated apples after storage in NA and CA conditions and after shelf life

Length of storage (days)	Storage conditions	Treatment	Activity of ACC oxidase ($nl \cdot g^{-1}$ $FW \cdot h^{-1}$)	
			After storage	After shelf life
Season 2001/2002				
120	NA	Untreated	398.16 c	751.74 b
		MCP	39.29 a	86.07 a
	CA	Untreated	561.44 d	892.36 b
		MCP	63.25 b	65.98 a
180	NA	Untreated	604.18 c	817.24 c
		MCP	111.55 b	526.36 b
	CA	Untreated	691.93 c	1039.10 c
		MCP	61.09 a	157.47 a
Season 2002/2003				
120	NA	Untreated	388.93 c	497.57 b
		MCP	32.65 a	59.29 a
	CA	Untreated	498.28 c	706.35 c
		MCP	67.93 b	73.93 a
180	NA	Untreated	499.08 c	479.53 c
		MCP	47.16 a	64.19 b
	CA	Untreated	887.91 d	581.16 c
		MCP	90.05 b	42.13 a

Note: see Table 1

The quality parameters of apples after harvest were as follow: a soluble solids content 10.8% and 11.8%, titratable acidity 0.357% and 0.402%, and flesh firmness of 82.1 N and 80.6 N, in years 2001 and 2002 respectively. Softening of apples is one of one of the most sensitive to ethylene ripening processes (Lelievre *et al*. 1997a). The application of 1-MCP delayed softening of 'Gala' apples stored in NA and CA conditions directly after storage and after 7 days of shelf life (Table 3), as has been shown by Lafer (2003) and Argenta *et al*. (2005). Similar effects of 1-MCP delaying fruit softening have been also observed for various apple cultivars: 'McIntosh', 'Delicious', 'Law Rome' (DeEll *et al*. 2005, Watkins *et al*. 2000), 'Empire' (DeEll *et al*. 2002, 2005,

Watkins *et al*. 2000), 'Cortland' (DeEll *et al*. 2002, Jóźwiak & Rutkowski 2001), 'Granny Smith' (Zanella 2003), 'Braeburn', 'Elstar' (Lafer 2003) and 'Anna'(Pre-Aymard *et al*. 2003), and for other climacteric fruits: apricots and plums (Dong *et al*. 2002), peaches and nectarines (Liguori *et al*. 2004) and avocado (Jeong *et al*. 2003).

Table 3. Flesh firmness of 1-MCP treated and untreated apples after storage in NA and CA conditions and after shelf life

Length of storage (days)	Storage conditions	Treatment	Flesh firmness (N)	
			After storage	After shelf life
Season 2001/2002				
120	NA	Untreated	74.01 a	73.45 a
		MCP	80.02 b	81.01 b
	CA	Untreated	76.40 a	75.32 a
		MCP	80.61 b	84.08 b
180	NA	Untreated	64.50 a	59.23 a
		MCP	75.74 b	79.24 c
	CA	Untreated	65.84 a	65.32 b
		MCP	81.40 c	80.22 c
Season 2002/2003				
120	NA	Untreated	66.87 a	63.13 a
		MCP	75.30 c	76.03 c
	CA	Untreated	68.87 b	66.23 b
		MCP	73.47 c	74.80 c
180	NA	Untreated	61.17 a	60.30 a
		MCP	72.67 b	76.10 b
	CA	Untreated	60.20 a	58.20 a
		MCP	74.03 b	74.17 b

Note: see Table 1

Taking into consideration the loss of firmness during storage 'Gala' apples characterized high storability. During 180 days of storage there were no positive effect of CA conditions compare to NA (except 120 days of storage in 2002/2003 season). Although there was a significant effect of 1-MCP for retention of firmness in both NA and CA conditions. The stronger effect of 1-MCP than controlled atmosphere on fruit firmness corresponds with the higher inhibition of ethylene production by 1-MCP treatment, and confirm the strong effect of 1-MCP on inhibiting ethylene action (Sisler & Serek 1997).

Storage conditions and 1-MCP treatment also influenced titratable acidity of stored apples in both years of experiment (Table 4). Regardless of the season and the length of storage period the highest loss of TA was observed for untreated apples stored in air. Application of 1-MCP after harvest allowed to keep TA of 'Gala' apples stored in air as well as CA conditions for untreated fruits. More often the highest titratable acidity was observed for 1-MCP treated apples stored under CA conditions. The beneficial effect of 1-MCP on reduction in loss

of titratable acidity was reported for 'Gala' (Argenta *et al*. 2005, Lafer 2003, Rutkowski & Jóźwiak 2001), 'Redchief Delicious' (Argenta *et al*. 2005), 'Anna' (Pre-Aymard *et al*. 2003), 'Granny Smith' (Zanella 2003), 'Braeburn', 'Elstar' (Lafer 2003) apples, peaches and nectarines (Ligouri *et al*. 2004), and plums (Dong *et al*. 2002).

Table 4. Titratable acidity in 1-MCP treated and untreated apples after storage in NA and CA conditions and after shelf life

Length of storage (days)	Storage conditions	Treatment	Titratable acidity (% of malic acid)	
			After storage	After shelf life
Season 2001/2002				
120	NA	untreated	0.251 a	0.239 a
		MCP	0.318 b	0.297 b
	CA	untreated	0.320 b	0.286 b
		MCP	0.329 b	0.321 c
180	NA	untreated	0.182 a	0.158 a
		MCP	0.284 b	0.276 c
	CA	untreated	0.287 b	0.255 b
		MCP	0.330 c	0.302 d
Season 2002/2003				
120	NA	untreated	0.298 a	0.282 a
		MCP	0.376 b	0.383 c
	CA	untreated	0.360 b	0.348 b
		MCP	0.377 b	0.368 bc
180	NA	untreated	0.246 a	0.229 a
		MCP	0.362 bc	0.357 c
	CA	untreated	0.349 b	0.330 b
		MCP	0.372 c	0.352 c

Note: see Table 1

Data presented in Table 5 shown that there was no clear effect of 1-MCP treatment on total soluble solids content in 'Gala' apples. In 2001/2002 season, treatment with 1-MCP had no influence on TSS in apples stored for 120 days, but after 180 days of storage the 1-MCP treated fruits had significantly higher TSS content than untreated ones. In the season 2002/2003, no statistical differences in TSS was observed in apples directly after storage. In both storage seasons, after shelf life the positive effect of 1-MCP on TSS was noticed, but only after 180 days the differences were statistically proved. DeEll *et al*. (2002) and Zanella (2003) reported that 1-MCP treatment not influenced TSS in apples.

Table 5. Total soluble solids in 1-MCP treated and untreated apples after storage in NA and CA conditions and after shelf life

Length of storage (days)	Storage conditions	Treatment	Total soluble solids (%)	
			After storage	After shelf life
Season 2001/2002				
120	NA	untreated	10.97 a	10.46 a
		MCP	11.09 a	10.91 ab
	CA	untreated	11.58 b	11.04 ab
		MCP	11.35 ab	11.29 b
180	NA	untreated	10.07 a	10.30 a
		MCP	11.39 b	10.96 b
	CA	untreated	11.24 b	10.60 ab
		MCP	11.89 c	11.61 c
Season 2002/2003				
120	NA	untreated	12.47 a	12.07 a
		MCP	12.53 a	12.50 ab
	CA	untreated	12.47 a	12.47 ab
		MCP	12.30 a	12.70 b
180	NA	untreated	12.23 a	11.60 a
		MCP	12.63 a	12.70 c
	CA	untreated	12.40 a	12.20 b
		MCP	12.47 a	12.57 c

Note: see Table 1

CONCLUSIONS

The results of the experiment indicate that the application of controlled atmosphere conditions and 1-MCP treatment are effective in delay of the ripening processes in 'Gala' apples by inhibiting the ethylene production. Internal ethylene concentration was significantly lower for the 1-MCP treated fruits, both directly after storage in NA or CA and after shelf life. 1-MCP treatment strongly inhibited ACC oxidase in 'Gala' apples following storage in air or controlled atmosphere. There was a positive effect of CA conditions and 1-MCP treatment on retention of firmness and titratable acidity of 'Gala' apples.

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WPŁYW WARUNKÓW PRZECHOWANIA I TRAKTOWANIA 1-MCP NA PRODUKCJĘ ETYLENU I JAKOŚĆ JABŁEK 'GALA'

Streszczenie

W dwóch kolejnych sezonach przechowalniczych 2001/2002 i 2002/2003 oceniano wpływ warunków przechowywania (normalna i kontrolowana atmosfera) i traktowania 1-MCP na produkcję etylenu i jakość jabłek odmiany 'Gala'. Po zbiorze jabłka zostały schłodzone do temperatury +1ºC i potraktowane 1-MCP w stężeniu 0,625 $\mu l \cdot L^{-1}$ przez 24 godziny. Traktowane i nietraktowane (kontrolne) jabłka były przechowywane przez 120 i 180 dni w chłodni w temperaturze 2±0,5°C, w normalnej (NA) i kontrolowanej atmosferze (KA) o składzie 2% O_2 i 2% CO_2. Bezpośrednio po wyjęciu owoców z chłodni i po dodatkowych 7 dniach przetrzymywania w temperaturze 18°C (symulowany obrót towarowy – SOT) mierzono stężenie etylenu w komorach nasiennych jabłek oraz oznaczano aktywność oksydazy ACC. W każdym z terminów analiz oceniano również jędrność owoców, zawartość ekstraktu i kwasowość miareczkową.

Pozbiorcze traktowanie owoców 1-MCP silnie hamowało aktywność oksydazy ACC w jabłkach przechowywanych przez 120 i 180 dni, zarówno w warunkach NA jak i KA. Wpływ ten potwierdził się również po okresie symulowanego obrotu towarowego. Nie zaobserwowano ograniczenia aktywności oksydazy w nietraktowanych jabłkach przechowywanych w warunkach KA w stosunku do owoców przechowywanych w NA, jednakże bezpośrednio po wyjęciu owoców z chłodni stężenie etylenu w komorach nasiennych jabłek przechowywanych w KA było niższe niż tych przechowywanych w NA. Stężenie etylenu w komorach nasiennych jabłek traktowanych 1-MCP było istotnie niższe niż w nietraktowanych. Zależność ta została statystycznie udowodniona zarówno dla owoców przechowywanych w warunkach NA jak i KA, bezpośrednio po wyjęciu jabłek z chłodni, jak i po okresie symulowanego obrotu towarowego.

Traktowanie jabłek odmiany 'Gala' preparatem 1-MCP ograniczało mięknięcie owoców i spadek kwasowości podczas przechowywania owoców w NA i KA. Nie stwierdzono powtarzalnego efektu traktowania na zawartość ekstraktu w owocach.