STORAGE QUALITY OF LOW ETHYLENE PRODUCING APPLES

Richard Colgan, John Stow, Annalisa Marchese, Sladjana Nidzovic and Mark Else

East Malling Research, East Malling, Kent ME19 6BJ, UK

(Received July 29, 2005/Accepted November 8, 2005)

ABSTRACT

The mechanism and storage potential of two low ethylene producing apple lines E19/13 and E55/55 were studied. Reduced ethylene production was partly attributed to a lowering in the activity of the enzyme ACC synthase (ACS). The presence of allelic forms of the ripening specific *ACS1* gene (*ACS1-2/2*) is known to cause low ACC synthase activity in apple. E19/13, E55/55 and one of their parents, 'Gloster 69' have an *ACS1-2/2* allelic constitution. However, ethylene production in E19/13 was significantly lower than in 'Gloster 69', despite sharing the same *ACS1-2/2* genotype. Other genes may contribute to the overall effect of low ethylene production in certain apple lines.

The storage potential of E19/13 and E55/55 apples was evaluated in air and in CA (1-3 O_2 %) conditions. Storage of E19/13 and E55/55 apples in air allowed for increased synthesis of two flavour volatiles, butyl and hexyl acetate, compared to storage in 1% O_2. Increasing the O_2 concentrations from 1% to 2-3% did not increase further the synthesis of butyl and hexyl acetates. Addition of hexanol to cortex samples from apples previously stored in 1% oxygen increased the synthesis of hexyl acetate in fruit which provides evidence that low oxygen storage reduces the availability of precursors for flavour volatile production. Only a limited reduction in fruit firmness was recorded in E19/13 and E55/55 apples after four months storage in either air or 1% O_2. Moreover, E55/55 failed to soften after a further three weeks shelf-life period at 20°C.

Key words: apple, ethylene, flavour, hexyl acetate, firmness, ACC synthase, *ACS1*

INTRODUCTION

Control of ethylene production is essential for the retention of fruit firmness during storage. This is often achieved through use of controlled atmosphere (CA) storage which is able to suppress ethylene production and extend the storage life of apples. Oxygen concentrations are often reduced to 1-2% during CA storage. However, this adversely affects the production of volatile flavour components.

In general, volatile compound production is highly dependent on the level of ripeness and is closely related to changes in respiratory rate and ethylene production. Esters are an important group of compounds that contribute to the flavour and aroma of ‘Cox’s Orange Pippin’ apples (Bartley et al., 1985; Williams and Knee, 1977). Volatile esters are grouped by their acid moiety: acetate, hexanoate, 2-methylbutyrate, butyrate, propanoate, octanoate or pentanoate. The principal acetate esters associated with apple flavour are ethyl acetate, butyl acetate, hexyl acetate and propyl acetate. Synthesis of acetate esters in ripening fruit is less affected by ethylene than other volatile esters (Fan et al., 1998). Moreover, butyl and hexyl acetates content of Cox type apples is influenced by season but is less affected by maturity at harvest or respiration rate during storage (Knee et al., 1990). However, synthesis of acetate esters is suppressed during storage in low concentrations of oxygen (1.25%) used in controlled atmosphere storage of apples (Bartley et al., 1985).

Exploitation of conventional apple breeding material with inherently low ethylene production capacity may allow apples to be stored in air or less stringent CA conditions while still maintaining good texture and maximising the synthesis of acetate ester production. Identifying the mechanism for low ethylene production in apples will enable markers to be developed for the selection of future apple lines which have a high storage potential. This paper explores the mechanism of low ethylene production in apples and investigates how apples respond in CA and air storage.

MATERIAL AND METHODS

Apples of the cultivars ‘Fiesta’ and ‘Gloster 69’, and two progeny from a ‘Fiesta’ x ‘Gloster 69’ cross, E19/13 and E55/55, were harvested on September 21, 2002. Approximately 3 kg of fruit was loaded into 4.5 L polypropylene pots and cooled overnight to 3.5°C before the pots were sealed and connected to a flow (1L kg^{-1} h^{-1}) of air. Four replicate pots per cultivar were used. Ethylene was measured using a UniCam 610-GC with an Alumina 100/120 column (1 m long, 0.6 mm internal diameter).

Extraction and assay of ACC synthase was performed according to the method of Yip et al. (1991). Eight replicates of each sample were extracted from each cultivar, at harvest and after 15 weeks of storage in air at 3.5°C.

Extraction of DNA from young leaves of ‘Fiesta’, ‘Gloster 69’, E19/13 and E55/55 apples was conducted using a DNA-CTAB extraction protocol (Doyle and Doyle 1987). Polymerase chain reaction (PCR) to identify *ACS1* allelic forms in cultivars and breeding selections was carried out according to Sunako et al. (1999).

Apples for volatile analysis were stored at 4°C in flowing streams of air and 1, 2 and 3% O_2 for eight months. They were then transferred to 20°C for seven days to promote the production of volatile flavour components. A 10-mm-diameter cylinder of cortex was extracted from the centre of each of five apples. After removing the epidermal layers, two five-mm-thick slices were cut from the ends and placed in a 20 mL glass syringe sealed with a silicone septum. Syringes were incubated at 30°C for 30 min. A 1 mL sample of the headspace was removed and injected into a GC fitted with an FID and a 10% Apiezon on 80-100 Chromasorb G-AW column (2.5 m long and 0.3mm internal diameter) column run at 170°C using nitrogen (33 mL min^{-1}) as the carrier gas.

The capacity of low ethylene producing fruit to synthesise hexyl acetate from hexanol was determined on five fruits per sample. 'Gloster 69', E55/55 and E19/13 apples were stored in 1% oxygen at 4°C for four months before being transferred to air at 20°C. Four, 5-mm- thick (10 mm diameter) slices of cortex were extracted from apples at 0, 7 and 14 days after transfer to 20°C; cortex samples were placed in 20 mL glass syringes containing either air or air + 3µL L hexanol. Syringes were sealed and incubated at 30°C for 30 min before a 1 mL sample of headspace was removed. The concentration of hexyl acetate was determined as above.

Apples for fruit firmness experiments were either stored in 1% O_2 or in air at 4°C for four months; afterwards apples were transferred to 20°C and firmness measurements were made after 24 hours, 7, 14 and 21 days after transfer to 20°C, using a motorised Lloyd-LRX fitted with an 11 mm Magnus Taylor probe.

RESULTS

Ethylene production of 'Fiesta' rose sharply immediately after harvest and remained stable at 4.5 nL g^{-1} h^{-1} for nine weeks (Fig. 1). Ethylene production in 'Gloster 69' and E55/55 was delayed until 46 days after harvest, and then increased to a maximum of 5.5 and 2.5 nL g^{-1} h^{-1}, respectively. Ethylene production in E19/13 apples remained very low throughout the storage period.

The activity of ACC synthase was higher in 'Fiesta' than the other three cultivars at harvest and increased after 15 weeks of air storage at 3.5°C (Tab. 1). Molecular markers for *ACS1* alleles identified differences in the ACC synthase 1 gene in the four apple cultivars (Fig. 2). 'Fiesta' was heterozygous (*ACS1-1/2*), and 'Gloster 69', E55/55 and E19/13 were all homozygous for *ACS1-2/2*.

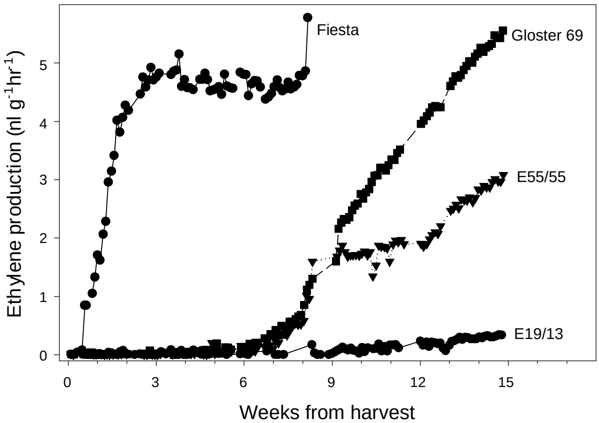


Figure 1. Ethylene production of 'Fiesta', 'Gloster 69', E55/55 and E19/13 apples stored in a flow of air [1 L kg^{-1} h^{-1}] at 3.5°C

T a b l e 1 . Maximum ACC synthase activity [nmoles ACC mg^{-1} protein] of apples at harvest and after fifteen weeks of air storage at 3.5°C

	'Fiesta'	'Gloster 69'	E55/55	E19/13
Harvest	482	108	148	68
Ex-store	1507	139	574	301

SED = 93.4 on 46 df

The firmness of 'Fiesta' declined by 20 N after four months storage in air (Tab. 2), but fruit stored in 1% O_2 had only softened by 8 N. Once at 20°C, 'Fiesta' from 1% O_2 and air storage softened to a similar rate. Air stored 'Gloster 69' fruits were 20 N firmer than 'Fiesta' apples on removal from storage but softened at a similar rate to 'Fiesta' when stored in air at 20°C. E55/55 apples remained very firm at 105 and 103 N after four months storage in air and 1% O_2, respectively. Moreover, E55/55 apples once at 20°C, softened very slowly. E19/13 apples did not soften during the initial four months storage in either air or CA, but after three weeks at 20°C air and CA stored fruit had softened by 27.8 N and 35 N, respectively.

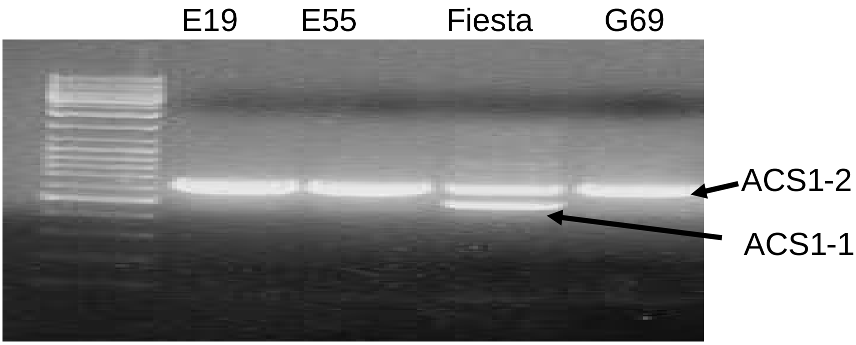
T a b l e 2 . Firmness [N] of apples removed from either air or 1% oxygen (3.5°C) in January 2004 and stored at 20°C for up to three weeks

Inspection	Storage	'Fiesta'	'Gloster 69'	E55/55	E19/13
Harvest*		87.0	92.8	117.7	91.6
24 hours	Air	67.4	87.4	105.6	94.1
	1% O_2	79.0	79.3	103.0	94.6
Week 1	Air	44.1	47.5	74.6	85.7
	1% O_2	54.9	46.4	107.0	79.4
Week 2	Air	45.1	38.1	93.2	84.1
	1% O_2	55.6	39.0	98.7	82.8

Week 3	Air	38.9	37.1	91.0	65.4
	1% O_2	38.4	39.1	103.5	59.6

*SED 3.97 on 36 df for harvest firmness data
SED 5.36 on 264 df. For storage x variety x inspection interaction

Figure 2. Analysis of the ACS1 allelic form in apple cultivars



In E55/55 and E19/13, two volatile flavour components, hexyl acetate and butyl acetate, were highest in apples stored in air (Tab. 3). Lowering the oxygen concentration in the storage environment to between 1 and 3 % significantly reduced production of hexyl acetate and butyl acetate in cortex samples. The production of hexyl acetate and butyl acetate did not increase when the concentration of oxygen was raised from 1 to 3%.

Table 3. Concentration of butyl and hexyl acetate [μL g^{-1} h^{-1}] in low ethylene producing apples stored at 3.5°C

Selection	Storage O_2%	Butyl acetate	Hexyl acetate
E55/55	1	5.14	0.77
	2	5.14	1.34
	3	3.70	0.72
	21	21.32	8.79
E19/13	1	1.44	0.52
	2	2.26	0.76
	3	1.64	0.46
	21	13.94	5.62

SED 0.43 on 32 df

The addition of hexanol to cortex samples increased hexyl acetate content in apples previously stored in 1% oxygen before being transferred to air storage at 20°C (Tab. 4). Increasing the length of time samples were stored at 20°C before analysis did not increase the hexyl acetate content of fruit. In this assessment, there was no difference in hexyl acetate content between the cultivars.

T a b l e 4. Concentration of hexyl acetate [μL g^{-1} h^{-1}] in apple cortex tissue supplemented with hexanol

Days at 20°C	0 days		7 days		14 days	
Hexanol added	no	yes	no	yes	no	yes
E19	0	3.69	0.04	3.50	0.38	4.52
E55	0.01	4.84	0.37	7.61	0.26	5.26
'Gloster 69'	0	3.37	0.05	3.85	0.08	3.56

SED =1.07 on 40 df

DISCUSSION

E55/55 and E19/13 apples have a restricted capacity to produce ethylene. This is partly caused by the reduced activity of ACC synthase. The presence of allelic forms of ACS1 gene in apple reduces ACC synthase activity and ethylene production (Sunako et al., 1999). Even though 'Gloster 69', E55/55 and E19/13 apples are all homozygous for the *ACS1-2* allele, they have different capacities for ethylene synthesis. Other genes that may contribute to reduced synthesis of ethylene are currently under study.

E19/13 and E55/55 soften less after transfer to 20°C than 'Fiesta' or 'Gloster 69', most likely because of reduced ethylene production. In E55/55 after transfer to 20°C, the synthesis of softening enzymes may be severely retarded, even though E55/55 can synthesize ethylene if given time. E55/55 was slightly firmer when stored in 1% oxygen than when stored in air alone. However, all E55/55 apples were very firm after three weeks of storage at 20°C. Long-term storage of E55/55 in air may be a practical option which does not compromise texture. E19/13 was softer than E55/55 after storage at 20°C, even though E19/13 produces very little ethylene. This is partly because of internal breakdown of the cortex.

In E55/55 and E19/13, hexyl acetate and butyl acetate synthesis was lower with storage at low concentrations of oxygen than with storage in air. Storage at low concentrations of oxygen limits oxidative processes such as the oxidation of long chain fatty acids, which provide a source of butanol and hexanol precursors (Bartley et al., 1985). The addition of hexanol to apple cortex stored under 1% O_2 increased the amount of hexyl acetate present in the cortex tissue and suggests that the terminal esterification of alcohols in apples is not affected by low levels of oxygen.

Acknowledgements. The authors are grateful to DEFRA (grant HH2606STF) for funding this work.

REFERENCES

Bartley I.M., Stoker P.G., Martin A.D., Hatfield S.G.S., Knee M. 1985. Synthesis of aroma compounds by apples supplied with alcohols and methyl esters of fatty acids. J. SCI. FOOD AGIC. 36: 567-574.

Doyle J.J., Doyle J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. PHYTOCHEM. BULL. 19(1): 11-15.

Fan X., Mattheis J.P., Buchanan D. 1998. Continuous requirement for ethylene for apple fruit volatile synthesis. J. AGRIC. FOOD CHEM. 46: 1959-1963.

Knee M., Hatfield S.G.S., Farman D. 1990. Sources of variation in firmness and ester content of 'Cox' apples stored in 2% oxygen. ANN. APPL. BIOL. 116: 617-623.

Sunako T., Sakuraba W., Senda M., Akada S., Ishikawa R., Niizeki M., Harada T. 1999. An allele of the ripening-specific 1-aminocyclopropane-1-carboxylic acid synthase gene (ACS1) in apple fruit with a long storage life. PLANT PHYSIOL. 119: 1297-1303.

Williams A.A., Knee M. 1977. The flavour of Cox's Orange Pippin apples and its variation with storage. ANN. APPL. BIOL. 87(1): 127-131.

Yip W-K., Dong, J.G., Yang S.F. 1991. Purification and characterization of 1-aminocyclopropane-1-carboxylate synthase from apple fruit. PLANT PHYSIOL. 95: 251-257.

JAKOŚĆ PRZECHOWALNICZA JABŁEK O NISKIEJ PRODUKCJI ETYLENU

Richard Colgan, John Stow, Annalisa Marchese, Sladjana Nidzovic i Mark Else

S T R E S Z C Z E N I E

Badano mechanizm i potencjał przechowalniczy dwóch linii jabłoni (E19/13 i E55/55) o niskiej produkcji etylenu. Obniżona produkcja etylenu wynikała częściowo z obniżonej aktywności enzymu, syntazy ACC (ACS). Wiadomo, że obecność allelicznych form genów specyficznych dla dojrzewania *ACSI* (*ASCI-2/2*) powoduje obniżoną aktywność syntazy ACC w jabłkach. E19/13, E55/55 i jedno z ich rodziców, 'Gloster' 69, zawierają allel *ACS1-2/2*. Jednakże produkcja etylenu w owocach E19/13 była znacząco niższa niż w owocach odmiany 'Gloster 69', pomimo posiadania wspólnego genotypu *ACSI-2/2*. U pewnych linii jabłoni do ogólnego efektu niskiej produkcji etylenu mogą przyczyniać się również inne geny.

Potencjał przechowalniczy jabłek linii E19/13 i E55/55 oceniano w warunkach normalnej i kontrolowanej atmosfery (1-3 O_2 %). Przechowywanie jabłek E19/13 i E55/55 w normalnej atmosferze przyczyniało się do zwiększonej syntezy dwóch zapachowych związków lotnych, octanu butylu i heksylu, w porównaniu do przechowywania w 1% O_2. Zwiększanie stężenia O_2 z 1 do 2-3% nie powodowało dalszego zwiększenia syntezy octanów butylu i heksylu. Dodanie heksanolu do wycinków z miąższu owoców przechowywanych wcześniej w 1% tlenu zwiększało syntezę octanu heksylu, co wskazuje na to, że przechowywanie w niskim stężeniu tlenu obniża dostępność prekursorów do produkcji związków lotnych. Po 4 miesiącach przechowywania zarówno w normalnej atmosferze, jak i w 1% O_2 stwierdzono jedynie nieznaczne obniżenie jędrności owoców E19/13 i E55/55. Ponadto E55/55 nie wykazywały mięknięcia po dalszych trzech tygodniach przetrzymywania w temperaturze 20°C.

Słowa kluczowe: jabłka, etylen, aromat, octan heksylu, jędrność, syntaza ACC, *ACS1*