Fluorescence, Conjugated Trienes, α-Farnesene and Storage Disorders in 'Abbé Fétel' Pears Cooled with Different Speeds and Treated with 1-MCP

M. Vanoli, M. Grassi, P. Eccher Zerbini[a] and A. Rizzolo
CRA-IAA, Agricultural Research Council - Food Technology Research Unit
Milan
Italy

Keywords: cooling speed, fluorescence, senescent scald, superficial scald

Abstract

'Abbé Fétel' pears are very prized in Italy, due to their non-melting, juicy texture and excellent flavour when ripe. However, they cannot be stored for long term in normal atmosphere (NA), because after 3-4 months they loose the ripening ability, while in controlled atmosphere (CA) they can be subject to senescent (soft) scald. 'Abbé Fétel' pears picked at two times were cooled to -0.5°C at different speeds (normal: immediately put at -0.5°C; slow: from 8°C decreasing 1°C d^{-1}), while monitored with fluorescence sensors (HarvestWatch™). Cooled fruit were treated with 300 ppb 1-MCP and stored in normal air or in controlled atmosphere (2% O_2 + 0.7% CO_2) at -0.5°C. After 4 and 6 months storage, fruit were analyzed immediately after storage and after 9 d of shelf life at 20°C for mass, skin colour and firmness. Fruit skin was extracted with hexane for analysis of α-farnesene and conjugated trienes (CTs) by spectrophotometric method. Cooling speed affected fruit fluorescence (Fα) and the effect was maintained throughout the storage. Fα rapidly increased both due to the fast decrease of temperature and to the decrease of oxygen partial pressure. During storage in CA and NA, Fα decreased more and earlier in fruit cooled slowly than in fruit exposed to normal cooling. In NA the decrease was gradual and steady, while in CA a quick decrease of Fα occurred after 4 (slow cooling) or 5 months storage (normal cooling). Among storage disorders, superficial scald was remarkably influenced by storage atmosphere, being almost fully controlled in CA. Senescent scald was induced especially in CA, and aggravated by slow cooling. The treatment with 1-MCP reduced both superficial and senescent scald, but was not fully effective due to delayed application. CTs and α-farnesene generally were higher after 4 than 6 months of storage, in NA than in CA, decreased with shelf life and were related to superficial scald.

INTRODUCTION

'Abbé Fétel' pears are very prized in Italy, due to their non-melting, juicy texture and excellent flavour when ripe. However they cannot be stored for long term in normal atmosphere (NA), because after 3-4 months they loose their ripening ability. In NA storage pears are sensitive to superficial scald, while in long term controlled atmosphere (CA) they are prone to senescent soft scald. The sesquiterpene α-farnesene and its oxidation products, conjugated trienes (CTs) are known to play a role in the development of superficial scald in pears as well as in apples (Whitaker, 2007). The senescent soft scald of pears is related to long term storage and to low oxygen in the atmosphere (Bertolini et al., 2002), and it is alleviated by 1-MCP treatment (Vanoli et al., 2007). It was hypothesised that it could be also related to the prompt cooling which is normally required for pear storage.

HarvestWatch™ (Satlantic Inc., Halifax. N.S., Canada) is a chlorophyll fluorescence sensor system, which produces a theoretical estimate of F_0 at zero irradiance, called Fα (Prange et al., 2003). Fα increases when O_2 concentration decreases below an O_2 threshold, and returns to approximately prestressed values if O_2 increases above the threshold, so allowing the detection of low-O_2 stress in chlorophyll containing fruit and

[a] p.e.zerbini@gmail.com

vegetables. The spike of Fα at the O_2 threshold indicates the transition of the fruit from aerobic to anaerobic metabolism. This system is generally used in steady state storage to dynamically set O_2 concentrations slightly above the tolerance level of the fruit (Dynamically Controlled Atmosphere, DCA; Prange et al., 2003; DeLong et al., 2004; Zanella et al., 2008). Here it was used to check how it may be affected by other storage factors, also during cooling and atmosphere set up.

This research aims at studying the fluorescence response of 'Abbé Fétel' pears to cooling speed and storage atmosphere, and the relationship of the latter to storage disorders and 1-MCP treatment, paying particular attention to CTs and α-farnesene contents.

MATERIALS AND METHODS

'Abbé Fétel' pears were picked on 19 (H1) and 27 of September 2006 (H2) in a commercial orchard near Modena, Italy. At harvest fruit were randomized in boxes and 30 fruit per harvest were analyzed for quality parameters (fruit mass, skin colour by Minolta Chromameter, starch hydrolysis by iodine test, fruit firmness, titratable acidity and soluble solids content (SSC)). On the day post harvest, half of the boxes were immediately cooled at -0.5°C (normal cooling), and the other half was put at 8°C, and cooled to -0.5°C with a temperature decrease of 1°C d^{-1} (slow cooling). On 9 October, H1 and H2 cooled fruit were treated with 0 (control) or 300 ppb 1-methylcyclopropene (1-MCP) at -0.5°C for 24h, and then stored at -0.5°C in normal air (NA) or in controlled atmosphere (CA, 2% O_2 + 0.7% CO_2). During normal and slow cooling and the subsequent 6 months of storage in CA and NA, samples of control H1 fruit were monitored with fluorescence FIRM™ sensors (HarvestWatch™, Satlantic, Canada). After 4 and 6 months' storage, fruit were analyzed immediately after storage (d0) and after 9 d of shelf life at 20°C (d9). Skin colour and firmness were measured on 12 fruit per treatment at d0 and d9. At d9 the analyses were carried out also on fruit affected by superficial scald and senescent scald, using the sound part of each fruit. On the same fruit, α-farnesene and conjugated trienes (CT281, CT258, CT269) were determined according to Zoffoli et al. (1998), after having pooled them into 3 groups of 4 pears per treatment. Storage disorders were examined after shelf life and scored for severity (1 = slight, 2 = moderate, 3 = severe). For superficial scald and senescent scald a severity index was computed by multiplying the number of fruit in each class by the score of the class and dividing their sum by the maximum possible score (as if all fruit had score 3).

Quality parameters, storage disorder incidence and severity indices were submitted to ANOVA considering harvest date, cooling speed, storage time and atmosphere, and 1-MCP treatment as factors. Data of CT281, CT258, CT269, α-farnesene, firmness and hue were submitted to PCA, analysing separately d0 and d9 data. Scores of principal components were analysed by ANOVA considering harvest date, cooling speed, storage time and atmosphere and 1-MCP treatment (d0, d9) and the status of fruit (sound, superficial scald, senescent scald) (d9) as factors (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

At harvest fruit had on average (mean ± s.e.): firmness, 51±10 N; SSC, 14±1°Brix; fruit mass, 286±62 g; starch hydrolysis, 7.1±1.7 (EUROFRU chart, 1-10 scores). Harvests were significantly different only for titratable acidity (H1, 3.3 meq 100 g^{-1}; H2, 3.6 meq 100 g^{-1}) and hue (H1, 100; H2, 98°).

Cooling speed affected fruit fluorescence: slow cooling did not cause remarkable changes in Fα during cooling, while normal cooling induced a rise in Fα. CA induced a further increase in Fα, but only in normal cooled fruit (Fig. 1). The differences in Fα between normal and slow cooling and due to CA setting were maintained throughout the most part of storage. During storage in CA and NA, Fα decreased more and earlier in fruit cooled slowly than in fruit exposed to normal cooling (Fig. 2). In NA the decrease was gradual and steady, while in CA a quick decrease of Fα occurred after 4 (slow cooling) or 5 months storage (normal cooling).

Storage disorders were almost absent after 4 months of storage and at d0 of post-storage shelf life, while they appeared after 6 months storage at d9 of shelf life. Superficial scald was remarkably influenced by storage atmosphere, being almost fully controlled in CA; in NA it was high and only partially controlled by 1-MCP (Table 1). Senescent scald was induced especially in CA, and aggravated by slow cooling, but its severity was only slightly reduced by the treatment with 1-MCP (Table 2).

Conjugated trienes and α-farnesene generally were higher in NA, in slow cooled fruit, after 4 months storage and at d0 of post storage shelf life. In fact α-farnesene after 4 months storage was 43 nmol cm^{-2} at d0 and 23 nmol cm^{-2} at d9, while after 6 months storage it was 27 nmol cm^{-2} at d0 and 24 nmol cm^{-2} at d9. After shelf life CTs and α-farnesene were significantly highest in fruit affected by superficial scald (which also showed the lowest hue and firmness) and lowest in those affected by senescent scald (data not shown).

In order to summarize results, Principal Component Analysis (PCA) was carried out on data of fruit pools analyzed for trienes, separately for d0 and d9 of shelf life. At d0, 89% of total variance was explained by the first two components (Table 3). CTs had the highest loadings in PC1, while α-farnesene, hue and firmness in PC2. At analysis of variance, PC1 scores were significantly higher after 4 months in NA stored fruit, in slow cooled and not treated fruit as compared respectively to 6 months, CA, normal cooling and 1-MCP treatment (Fig. 3). PC1 was much lower in 1-MCP treated fruit if cooled normally than if cooled slowly. PC2 was higher in H1 fruit, after 4 months storage, in CA, in normal cooling and 1-MCP treated fruit. The effect of 1-MCP treatment was greater in CA than in NA. After shelf life (d9) the two principal components explained 79% of total variance. The highest loadings in PC1 were those of CTs and α-farnesene (positive) and hue (negative), while firmness had the highest loading in PC2 (Table 3). Scores of both PCs were higher after 6 months and in not 1-MCP-treated fruit (Fig. 4). PC1 was highest in fruit affected by superficial scald, and lowest in those affected by senescent scald. The opposite was true for PC2. The 1-MCP treatment reduced the scores of both PCs, indicating that in fruit affected by superficial and senescent scald, 1-MCP was associated to a greener hue and to lower concentrations of CTs and α-farnesene, as expected, but also to lower firmness.

The data confirm that CTs and α-farnesene are related to superficial scald. The treatment with 1-MCP reduced both superficial and senescent scald, but it was not fully effective because its application was delayed in order to wait for the slow cooling. Analyses of the fluorescence signals showed that fruit fluorescence is not only affected by atmospheric composition, but also by temperature. So a rapid rise in fluorescence may be due both to a decrease of oxygen partial pressure and to a decrease of temperature. The changes in Fα that occurred at the beginning of storage are maintained during storage, without apparent relation to storage disorders, until other, long term effects occur. The long term reduction of Fα is probably due to chlorophyll breakdown, which results in fruit yellowing, while the long term increase, like that shown by CA, normal cooled fruit, may be thought of as an increase in stress, perhaps related to the climacteric peak.

CONCLUSIONS

The relation between superficial scald and CTs and α-farnesene was confirmed. CA fully controlled superficial scald, while senescent scald was induced by CA and aggravated by slow cooling.

Both superficial and senescent scald were reduced by the 1-MCP treatment. The need for a prompt 1-MCP treatment was confirmed, as this late treatment was only partially effective.

Fluorescence response was increased both by decreasing temperature and by decreasing oxygen partial pressure. This should be taken into account when using fluorescence sensors for Dynamically Controlled Atmosphere.

Further research is needed to elucidate the long term effects on fluorescence response.

Literature Cited

Bertolini, P., Guizzardi, M. and Casadei, C. 2002. Influence of calcium and oxygen levels on soft scald of stored 'Abbé Fétel' pears. Acta Hort. 596:851-856.

DeLong, J.M., Prange, R.K., Leyte, J.C. and Harrison, P.A. 2004. A new technology that determines low-oxygen thresholds in controlled-atmosphere-stored apples, HortTechnol. 14:262-266.

Prange, R.K., DeLong, J.M., Harrison, P.A., Leyte, J.C. and Mclean, S.D. 2003. Oxygen concentration affects chlorophyll fluorescence in chlorophyll-containing fruit and vegetables. J. Am. Soc. Hort. Sci. 128:603-607.

Vanoli, M., Rizzolo, A., Grassi, M. and Eccher Zerbini, P. 2007. Storage disorders and quality in 'Abbé Fétel' pears treated with 1-methylcyclopropene. Proc. Intern. Congress "Novel approaches for the control of postharvest diseases and disorders", Bologna, Italy, 3-5 May 2007. p.269-277.

Whitaker, B.D. 2007. Oxidation products of α-farnesene associated with superficial scald development in D'Anjou pear fruits are conjugated trienols. J Agric. Food Chem. 55:3708-3712.

Zanella, A., Cazzanelli, P. and Rossi, O. 2008. Dynamic controlled atmosphere (DCA) storage by the means of chlorophyll fluorescence response for firmness retention in apple. Acta Hort. 796:77-82.

Zoffoli, J.P., Richardson, D., Chen, P. and Sugar, D. 1998. Spectrophotometric characterization of superficial and senescent scald in pear fruits relative to different stages of maturity. Acta Hort. 475:543-558.

Tables

Table 1. Severity index for superficial scald: mean (s.e.).

	CA	NA	1-MCP		mean
			0 ppb	300 ppb	
4 months	0.000 (0)	0.034 (0.006)	0.021 (0.007)	0.011 (0.003)	0.016 (0.004)
6 months	0.019 (0.008)	0.294 (0.024)	0.191 (0.043)	0.128 (0.035)	0.161 (0.028)
mean	0.009 (0.004)	0.168 (0.027)	0.106 (0.026)	0.070 (0.020)	

Table 2. Severity index for senescent scald: mean (s.e.).

		4 months				6 months			
		CA		NA		CA		NA	
Cooling:	Normal	0.002	(0.002)	0.006	(0.003)	0.340	(0.077)	0.075	(0.037)
	Slow	0.105	(0.024)	0.001	(0.001)	0.565	(0.044)	0.008	(0.005)
1-MCP:	0 ppb	0.055	(0.028)	0.004	(0.003)	0.550	(0.043)	0.038	(0.030)
	300 ppb	0.052	(0.023)	0.003	(0.002)	0.357	(0.084)	0.045	(0.028)
mean		0.054	(0.018)	0.003	(0.002)	0.460	(0.051)	0.041	(0.020)

Table 3. Principal Component Analysis at day 0 and day 9: eigenvectors, eigenvalues and proportion of variance of the two first components (PC1 and PC2).

		Day 0		Day 9	
		PC1	PC2	PC1	PC2
Eigenvectors:	α-Farnesene	0.290	0.543	0.451	0.067
	CT258	0.513	0.060	0.479	-0.087
	CT269	0.514	0.090	0.509	-0.087
	CT281	0.493	0.136	0.448	-0.040
	Firmness	-0.214	0.626	0.016	0.973
	Hue	-0.316	0.531	-0.327	-0.177
Eigenvalue		3.63	1.71	3.70	1.03
Proportion of variance		0.60	0.29	0.62	0.17
Cumulative proportion		0.60	0.89	0.62	0.79

Figures

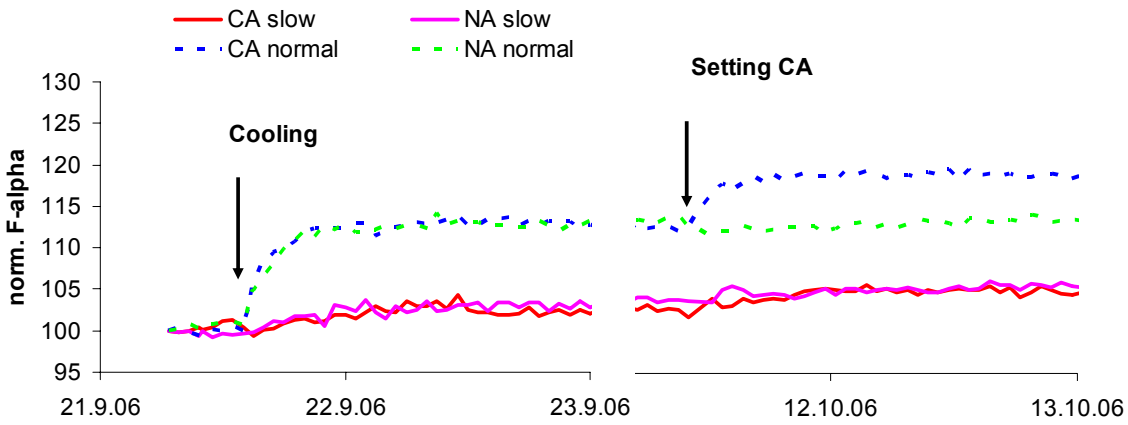

Fig. 1. Fluorescence as normalized Fα (100= at beginning of experiment) in pear fruit after beginning of cooling (left arrow) and of setting CA (right arrow).

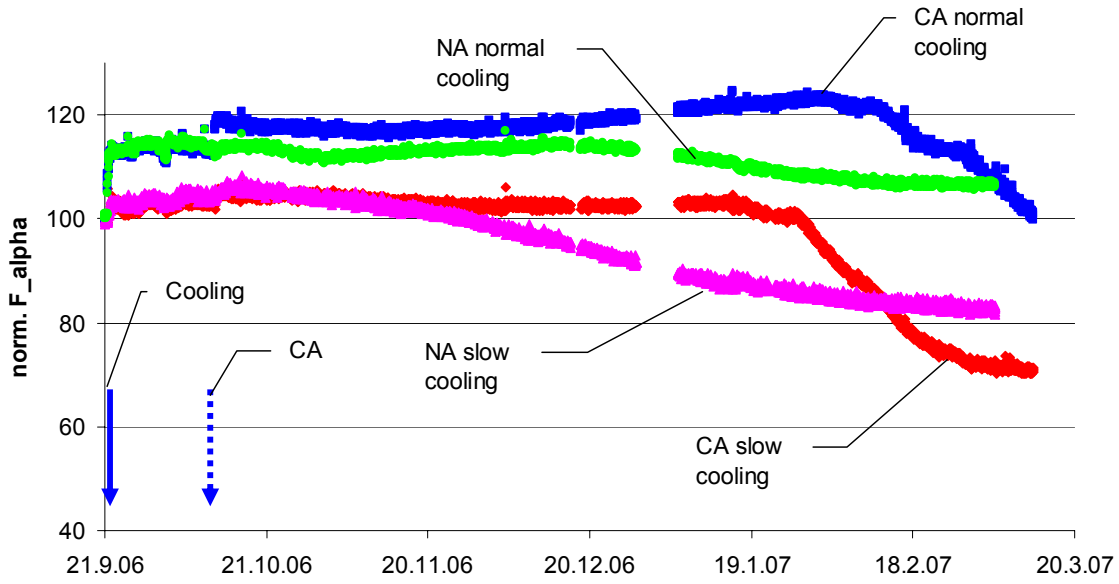

Fig. 2. Fluorescence as normalized Fα (100 = at beginning of experiment) during storage in relation to cooling method and storage atmosphere.

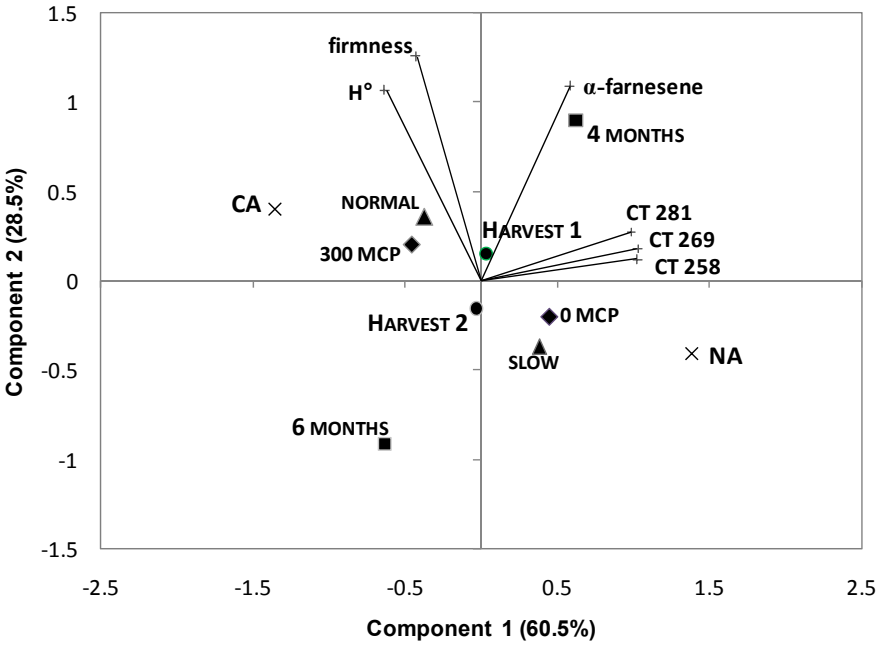

Fig. 3. Principal component analysis at d0 of post storage shelf life. Biplot of variable loadings and mean scores of the main factors. All main factors are significant at ANOVA, except for harvest on PC1.

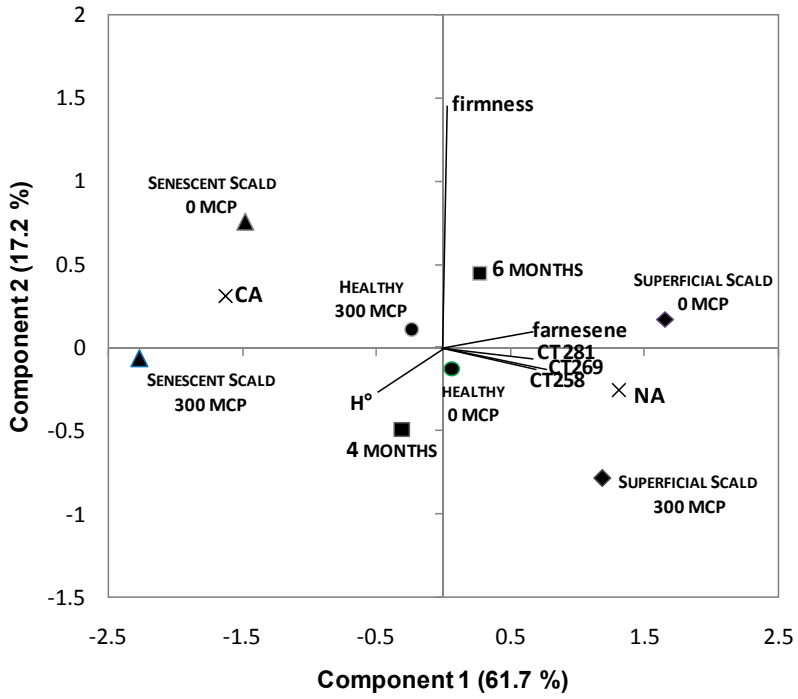

Fig. 4. Principal component analysis at d9 of post storage shelf life. Biplot of variable loadings and mean scores of the main factors significant at ANOVA, and of interaction 1-MCP × health status of fruit.