A model to predict ripening capacity of ‘Bartlett’ pears (*Pyrus communis* L.) based on relative expression of genes associated with the ethylene pathway

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Abstract

Resistance to ripening in European pears depends on various preharvest and postharvest factors. Exposure to cold temperatures has been demonstrated to condition pears to produce endogenous ethylene and ripen. Mature green ‘Bartlett’ pears were exposed to 0, 5, or 10 °C for 2 to 14 d to induce different rates of ripening. At higher temperatures, expression of ethylene biosynthesis genes (*ACS1a* and *ACO*) and ethylene receptor genes (*ETR2*, *ERS1a*, and *ETR1a*) increased, while *CTR1* expression decreased. Multiple linear regressions between relative expression of these genes after 0 d and fruit firmness after 6 d during ripening at 20 °C were conducted. Using K-fold cross validation and conventional validation, it appears that expression of *ACO* could be utilized as an early predictor of pear ripening capacity.

Keywords: European pears, firmness, low temperatures, ripening prediction.

- Expression of ACO after 0 d can be used to predict fruit firmness after 6 d of ripening.

1. Introduction

European pears develop a buttery and juicy texture when ripened off the tree (Murayama et al., 1998). However, most European pear fruit (*Pyrus communis* L.) have some resistance to ripening that can induce non-uniform ripening after harvest (Villalobos-Acuna and Mitcham, 2008). Although ‘Bartlett’ pears are less resistant to ripening compared to winter pears such as ‘Comice’ and ‘d’Anjou’ (Villalobos-Acuna and Mitcham, 2008), early season ‘Bartlett’ pears usually fail to ripen uniformly (Hansen, 1939) and sometimes stay > 80 N after 9 d (Agar et al., 2000b). Ethylene treatment (e.g., 100 $\mu\text{L L}^{-1}$ at 20 °C for 24 h) can be used to induce and speed up the ripening process (Agar et al., 2000a, 2000b; Hansen, 1939; Wang et al., 1971). This treatment can be replaced by low temperature exposure (i.e., -1 to 10 °C) (Villalobos-Acuna and Mitcham, 2008). In this low temperature range, intermediate temperatures (5 to 10 °C) have been reported to induce faster ripening more quickly than lower temperatures (-1 to 0 °C) (Agar et al., 2000b; Sugar and Basile, 2009).

Cold treatments hasten fruit ripening in European pears by triggering the ethylene pathway (Blankenship and Richardson, 1985; Villalobos-Acuna and Mitcham, 2008). Enzyme activities of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO), as well as ethylene production of ‘Passe-Crassane’ pears increased after three months at 0 °C (Lelievre et al., 1997). Among multiple ACS genes in ‘Passe-Crassane’ pears, only *Pc-ACS1a* showed an increase in expression after 80 d at 0 °C (El-Sharkawy et al., 2004); the ethylene receptor *Pc-ETR1a* transcript was also up-regulated at 0 °C (El-Sharkawy et al., 2003). In ‘Bartlett’ pears, ACS and ACO enzyme activities increased during ripening at 20 °C after 5 or 10 °C conditioning treatments; however significant increases in these enzyme activities were only observed following a few days of ripening at 20 °C after the fruit had been transferred from the cold treatments (Agar et al., 2000b).

The expression of genes associated with ethylene biosynthesis and signal transduction has not been assessed following cold exposure in ‘Bartlett’ pears. This study was conducted to examine the expression of genes associated with ethylene biosynthesis (*ACS1a*, *ACO*) and signal transduction (*ETR1a*, *ETR2*, *ERS1a*, and *CTR1*), comparing the effect of 0 °C and intermediate temperature (5 and 10 °C) treatments. Additionally, given that current maturity indices such as fruit firmness and skin color are unreliable indicators of pear fruit ripening rate (Agar et al., 2000a), we also investigated if the expression of ethylene-associated genes can be used as an early predictor of fruit ripening capacity. In this study, we define full ripening capacity as the ability of pear fruit to soften to ≤ 20 N after 6 d at 20 °C.

2. Materials and methods

2.1. Plant materials

Mature green early season 'Bartlett' pear fruit, average 82 N firmness and 65 mm average diameter were harvested in Sacramento County, California at the initiation of commercial harvest (July 19, 2010). Pear fruit were sorted to remove sunburned, damaged or defective fruit, and randomized before assignment to temperature treatments. Fruit were cold-treated at 0, 5, or 10 °C for 2, 5, 8, 11, or 14 d (14 d treatment was used only at 0 °C) (Table 1). Similar temperature conditioning experiments with 0 and 10 °C were repeated in 2012, 2013, and 2014 (Table 1). In 2012 and 2013, separate sets of fruit were also treated with 100 $\mu\text{L L}^{-1}$ ethylene at 20 °C for 12, 18, or 24 h (Table 1). The control received no cold or ethylene exposure. After treatment, fruit were then transferred to 20 °C and 90 % relative humidity for ripening. Each measurement described below was obtained from three biological replicates with six fruit per replicate.

Table 1: Summary of temperature and ethylene conditioning treatments applied over four years of study to enhance pear fruit’s ability to ripen.

Year	Treatment	
	Low temperature	Ethylene

	0 °C	5 °C	10 °C	Ethylene
2010	2, 5, 8, 11, or 14 d	2, 5, 8, or 11 d	2, 5, 8, or 11 d	NA
2012	5, 8, 11, 14, or 17 d	2, 5, 8, or 11 d	2, 5, 8, or 11 d	12, 18, or 24 h
2013	8, 11, or 14 d	NA	3, 5, or 7 d	12, 18, or 24 h
2014	3, 6, 9, 12, or 15 d	NA	3, 6, or 9 d	NA

NA: not applicable

2.2. Ripening evaluation

The rate of fruit softening at 20 °C was monitored by measuring firmness on opposite sides of each fruit after removing a section of skin after 0, 3, 6, and 9 d, using a Güss FTA Penetrometer fitted with an 8 mm probe (Güss, Strand, Western Cape, South Africa). Ethylene production rate was determined on headspace gases produced by six fruit per replicate after 0, 2, 4, 6, 8, and 10 d, using flame ionization gas chromatography (Model Carle AGC-211, EG&G Chandler Engineering, Tulsa, OK.) as described by Villalobos-Acuna et al. (2010).

2.3. Gene expression analysis

Gene expression analysis was conducted using a method from Nham et al. (2015). Peel tissues were collected from fruit at harvest (control) and from fruit that had been warmed to 20 °C for 3 h following the completion of each cold treatment or each ethylene treatment (0 d). Total RNA was isolated using the Qiagen RNeasy Plant Mini Kit (Qiagen, Netherlands) and treated with DNase I (Roche, Switzerland). RNA concentration and integrity were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, MA). cDNA was synthesized using 1 µg total RNA, using Superscript™ III First Strand Synthesis Systems for RT-PCR Systems (Invitrogen, CA). Relative gene expression was examined using SYBR Green PCR Master Mix and 7300 Real Time PCR System (Applied Biosystems, CA). *Ef1alpha* was chosen as the optimal internal control gene after testing with *18s*, *26s*, *beta-actin*, and *tubulin1*. Primers for tested

genes (*ACS1a*, *ACO*, *ETR1a*, *ETR2*, *ERS1a*, and *CTR1*) were designed using Primer 3 (Untergasser et al., 2012) (Table 2). Relative expression (RE) was presented as $2^{-\Delta Ct}$.

Table 2: Primers used in real-time PCR.

Name		Oligonucleotide sequence (5'-3')	NCBI Accession number and Note
<i>Pc-EF1alpha</i>	F	GGTGTGAAGCAGATGATTTG	AY338250
	R	TCACCCTCAAACCCAGATAT	
<i>Pc-ACS1a</i>	F	TCCTTTCCACACTTCTGTCTTACAGC	X87112 El-Sharkawy et al. (2004)
	R	GGGTAGGAATAAGAAGAACTTCGCC GG	
<i>Pc-ACO</i>	F	GTTTTACAACCCAGGCGATG	AJ504857
	R	TTCAAATCTTGGCTCCTTGG	
<i>Pc-ETR1a</i>	F	CGAGGCCATGTAAAGACAGG	AF386509
	R	GAAGGGTACACCGAGACTGC	
<i>Pc-ETR2</i>	F	AGTGCTGACGAAGGTGTGTG	HM561909
	R	CCAATGCTGGATTTTGGTC	
<i>Pc-ERS1a</i>	F	CAACAACACACCAGGTCCAG	AF386515
	R	TCAAAATCCCAGGTTGAGC	
<i>Pc-CTR1</i>	F	GATCAAAGCTCCCGTCACTC	HM156629
	R	GGCCATACCCGAGACTATGA	

2.4. Experimental design, model building and validation

The experiment was a completely randomized factorial design. Linear regression analysis or multiple linear regression analysis was used to predict the firmness of individual replicates after 6 d during ripening from the average RE of one or multiple genes across three replicate trials. Two methods of model building and validation were conducted. In the first method, fruit firmness and gene expression of six genes from 2010 and 2014 data (57 data points) were combined for the model training and validation. K-fold cross-validation was carried out with $k = 3$ (38 randomized data points were used as the training set, 19 remaining data points formed the test set) and $k = 19$; each run was repeated 100 times. In the second method,

2010 data (36 data points) were used as the training set and 2014 data (19 data points) were utilized to validate. For the gene *ACO*, 69 data points from 2012 and 2013 were also used to validate. These regression and validation analyses were performed on R 2.15.0 (The R Core Development Team, 2013; RStudio i386-pc-mingw32/i386 platform). The Bootstrap package (<https://cran.r-project.org/web/packages/bootstrap/index.html>) was used for the K-fold cross-validation.

3. Results and discussion

3.1. *Fruit ripening capacity development by low temperature conditioning*

Ripening capacity developed faster when the conditioning temperature increased from 0 °C to 10 °C (Figure 1). Without low temperature conditioning, the control fruit, which were transferred immediately to 20 °C after harvest, softened to 15.8 N after 12 d (Fig. 1A). Fruit slowly developed ripening capacity following exposure to 0 °C; after 14 d at 0 °C, fruit softened to 17.4 N after 6 d of ripening at 20 °C (Fig. 1E). Conditioning the fruit for 5 d at 5 or 10 °C promoted full ripening with fruit firmness of 14.2 and 10.2 N, respectively, after 6 d at 20 °C (Fig. 1B). In addition, higher and earlier peaks in ethylene production rate were observed in the fruit that softened more quickly (Fig. 1F-J). These results confirmed the effect of low temperatures on the development of fruit ripening capacity and ethylene production, which have been shown previously in ‘Bartlett’ (Agar et al., 2000b; Villalobos-Acuna and Mitcham, 2008) and in ‘d’Anjou’ (Sugar and Einhorn, 2011) pears.

Fig. 1. Firmness and ethylene production at harvest (AH) and during ripening at 20 °C after cold treatment (results in the 2010 experiments). Fruit were held at 0, 5, or 10 °C for 2, 5, 8, 11, or 14 d. Control: Fruit not subjected to cold treatment. During ripening at 20 °C, firmness was measured every 3 d; ethylene was measured every 2 d. Error bars indicate standard error of the means.

3.2. Expression of genes encoding ethylene biosynthesis and signal transduction

To understand the effect of different cold temperature treatments on ethylene pathways, expression of *Pc-ACS1a*, *Pc-ACO*, *Pc-ETR1a*, *Pc-ERS1*, *Pc-ETR2*, and *Pc-CTR1* was examined on samples collected at harvest and after low temperature treatments (Fig. 2A). We also chose two treatments, 0 °C for 14 d and 10 °C for 5 d, which had induced full ripening capacity, to evaluate expression of these ethylene genes during ripening (Fig. 2B). Exposure to 10 °C for ≥ 5 d resulted in increased expression of ethylene biosynthesis genes (*Pc-ACS1a* and *Pc-ACO*) and there was a slight up-regulation of these genes following exposure to 5 °C for ≥ 5 d. These results are in agreement with previous research in ‘Bartlett’ pears where enzyme activities of ACS and ACO were higher following exposure to 10 °C than to 5 °C (Agar et al., 2000b). Transcript abundance of *Pc-ACS1a* remained low and unchanged following 0 °C exposure (Fig.

2A) and started to increase after 6 d of ripening at 20 °C following exposure to 0 °C for 14 d (Fig. 2B). Meanwhile, an increase in expression of *Pc-ACO* was recorded as early as after 11 d following 0 °C exposure (Fig. 2A).

In addition to ethylene biosynthesis genes, expression of ethylene receptors (*Pc-ETR1a*, *Pc-ETR2*, and *Pc-ERS1a*) were examined following low temperature treatments and during fruit ripening (Fig. 2). These genes were up-regulated by the intermediate temperatures (10 °C and 5 °C) but showed unclear trends in the 0 °C treatments (Fig. 2A). The expression of *Pc-ETR1a* and *Pc-ETR2* also increased during ripening at 20 °C of fruit from the treatments that induced full ripening capacity (0 °C for 14 d or 10 °C for 5 d), as well as in the control fruit (Fig. 2B), although with slightly different patterns. The expression of *Pc-ETR1a* agrees with what was found in 0 °C-treated ‘Passe-Crassane’ pears (El-Sharkawy et al., 2003); however, in the same study, *Pc-ETR2* and *Pc-ERS1a* showed no changes during the 0 °C treatment and only increased during ripening. The earlier increase in transcript abundance of *Pc-ETR2* and *Pc-ERS1a* in ‘Bartlett’ pears compared to ‘Passe-Crassane’ pears after low temperature conditioning suggests that the transcription of genes encoding these ethylene receptors is more responsive to cold treatments in ‘Bartlett’ pears than in ‘Passe-Crassane’ pears; this may be the reason why it takes shorter cold exposure to induce ripening in ‘Bartlett’ pears than in ‘Passe-Crassane’ pears.

In contrast to the up-regulation of genes associated with ethylene biosynthesis and perception, transcript abundance of *Pc-CTR1* decreased after 2 d of conditioning at 5 and 10 °C, respectively (Fig. 2A). The decrease in expression of this gene in fruit conditioned at 0 °C began at 11 d. As *CTR1* serves as a negative regulator of ethylene response (Kieber et al., 1993), the decrease of *Pc-CTR1* during cold treatments in our data indicates the involvement of this gene in regulating the ethylene pathway at low temperatures, which consequently facilitated ethylene production and faster ripening. Additionally, during fruit ripening, the expression of *Pc-CTR1* increased in the control fruit after 3 d and 6 d before returning to low levels, while the expression of this gene remained low and relatively stable in the other

treatments (0 °C for 14 d and 10 °C for 5 d) (Fig. 2B), likely facilitating faster ripening. *Pc-CTR1* expression in ‘Passe-Crassane’ pears was found to slightly decrease within the first 20 d of a 0 °C treatment and increased at 15 d during ripening at 20 °C and then remained stable for 10 d (El-Sharkawy et al., 2003). In ‘Passe-Crassane’ pears, this increase in *Pc-CTR1* expression coincided with the onset of the climacteric production of ethylene and was hypothesized to temper the ethylene response (El-Sharkawy et al., 2003). In our study with ‘Bartlett’, an increase in *Pc-CTR1* expression occurred only in ripening control fruit. An increase in *CTR1* during fruit ripening has also been observed in kiwifruit, where expression of *Ad-CTRs* increased after 28 and 56 d of shelf-life at 20 °C after harvest, when the fruit was softening (Yin et al., 2009).

Fig. 2. Relative expression (RE) of *Pc-ACS1a*, *Pc-ACO*, *Pc-ETR1a*, *Pc-ETR2*, *Pc-ERS1a*, and *Pc-CTR1* in pear fruit A) at harvest (AH) and during exposure to low temperatures (0, 5, or 10 °C) for 2 to 14 d and B) during ripening of fruit from the control and two cold treatment protocols

inducing full ripening capacity (results in the 2010 experiments). Error bars indicate standard error of the means. The inserted figures indicate the magnification of the figure portion including gene expression at small values.

3.3. Model to predict fruit firmness after 6 d of ripening at 20 °C and validation

Method 1: Data of 2010 and 2014 were combined for the training and validation set.

Multiple linear regression analyses were performed between the relative expression (RE) of six genes after cold conditioning (0 d) and the $\log_2$ of fruit firmness (LogF) after 6 d of ripening at 20 °C. RE_{ACO} had the largest correlation with fruit firmness (Table 3); a simple linear regression between *Pc-ACO* expression and firmness (Model 3: $\text{LogF} = a_1 + a_2\text{RE}_{\text{ACO}}$) generated an R^2 of 0.819 (Table 4). When all six genes were integrated into the model (Model 1: $\text{LogF} = a_1 + a_2\text{RE}_{\text{ETR1a}} + a_3\text{RE}_{\text{ETR2}} + a_4\text{RE}_{\text{ERS1a}} + a_5\text{RE}_{\text{CTR1}} + a_6\text{RE}_{\text{ACO}} + a_7\text{RE}_{\text{ACS1a}}$), the R^2 increased to 0.866 (Table 4). Using 0.008 as the adjusted significance level (Bonferroni correction), the multiple regression analysis showed that RE_{ACO} and RE_{ACS1a} were significant predictors (Table 5). Using the two significant predictors in the multiple regression analysis (Model 2: $\text{LogF} = a_1 + a_2\text{RE}_{\text{ACO}} + a_3\text{RE}_{\text{ACS1a}}$), an R^2 of 0.863 was obtained (Table 4). Among the three models, Model 2 showed the highest adjusted R^2 and cross-validation R^2 (Table 4). The large impact of the presence of *ACS1a* on the model's significance is reasonable because ACS is generally considered to be the rate-limiting factor in the ethylene biosynthetic pathway (Harada et al., 2000; Lau et al., 1986).

Table 3: Pearson correlations between variables in the multiple linear regression models using 2010 and 2014 data.

	RE _{ACO}	RE _{ACS1a}	RE _{ETR1a}	RE _{ETR2}	RE _{ERS1a}	RE _{CTR1}	LogF
RE _{ACO}	1.00	0.77	0.37	0.80	0.77	-0.56	-0.90
RE _{ACS1a}	0.77	1.00	0.74	0.76	0.83	-0.70	-0.83

RE _{ETR1} _a	0.37	0.74	1.00	0.57	0.70	-0.73	-0.47
RE _{ETR2}	0.80	0.76	0.57	1.00	0.90	-0.52	-0.74
RE _{ERS1a}	0.77	0.83	0.70	0.90	1.00	-0.61	-0.76
RE _{CTR1}	-0.56	-0.70	-0.73	-0.52	-0.61	1.00	0.59
LogF	-0.90	-0.83	-0.47	-0.74	-0.76	0.59	1.00

Table 4: Regression models to predict firmness after 6 d of ripening based on relative expression of ethylene genes after 0 d of ripening, and validation based on 2010 and 2014 data.

	Model (2010 and 2014)	RMSE	R ²	R ² adj	R ² Cross Validation [†]	
					k=3	k=19
1	$\text{LogF} = 2.394 + 0.009\text{RE}_{\text{ETR1a}} + 0.112\text{RE}_{\text{ETR2}} - 0.002\text{RE}_{\text{ERS1a}} - 0.013\text{RE}_{\text{CTR1}} - 0.231\text{RE}_{\text{ACO}} - 0.133\text{RE}_{\text{ACS1a}}$	0.196	0.866	0.850	0.803	0.822
2	$\text{LogF} = 2.205 - 0.217\text{RE}_{\text{ACO}} - 0.115\text{RE}_{\text{ACS1a}}$	0.200	0.863	0.858	0.845	0.848
3	$\text{LogF} = 3.524 - 0.302\text{RE}_{\text{ACO}}$	0.264	0.818	0.815	0.806	0.809

p-value of all models $\leq 2e-16$; RMSE: Root mean squared error; adj: adjusted; [†]: R² is the mean of all values obtained from 100 resamplings

Table 5: Coefficients of Model 1

Coefficient s	Estimate	SE	p- value
(Intercept)	2.394	1.485	0.113
RE _{ETR1a}	0.009	0.078	0.913
RE _{ETR2}	0.112	0.160	0.487
RE _{ERS1a}	-0.002	0.165	0.993
RE _{CTR1}	-0.013	0.139	0.925
RE _{ACO}	-0.231	0.044	0.000
RE _{ACS1a}	-0.133	0.045	0.005

Method 2: Data of 2010 were used as the training set; data of 2014, as well as 2012 and 2013 for RE of ACO only, formed the validation set.

Using only the data from 2010, RE_{ACO} also showed the largest correlation with fruit firmness (Table 6) with an R^2 of 0.870 (Table 7). A higher R^2 value (0.928) was produced from the multiple regression analysis performed on RE of all six genes combined (Model 4, Table 7). In contrast to the results in Method 1, using the same threshold for p-value (0.008, Bonferroni correction) the multiple regression analysis showed that RE_{ETR1a}, RE_{ERS1a}, and RE_{CTR1} were each significant predictors and the remaining predictors, RE_{ACO}, RE_{ACS1a}, and RE_{ETR2}, were each insignificant (Table 8). The high collinearity among predictors such as between RE_{ACO} and RE_{ACS1a} may impact the p-value of these predictors in the multiple regression model (Table 6). When the three significant predictors were included in the multiple regression analysis (Model 5: $\text{LogF} = a_1 + a_2\text{RE}_{\text{ETR1a}} + a_3\text{RE}_{\text{ERS1a}} + a_4\text{RE}_{\text{CTR1}}$), ANOVA determined no significant difference (p-value = 0.305) between Model 4 (six genes) and Model 5 (three genes). We investigated positions of *ETR1a*, *ERS1a*, *CTR1*, *ACO*, and *ACS1a* in the published genome (Chagne et al., 2014) for a possible genetic relationship. However, none of these genes were found on the same scaffold (data not shown). Additionally, similar to the results of Method 1, the increased root mean squared error (RMSE) in the reduced multiple regression model and in the RE_{ACO} model suggests the trade-off when fewer genes were used to predict firmness (Table 4, 7).

The three models established by the 2010 data were validated using the 2014 data. The ACO model (Model 6) showed better prediction of fruit firmness after 6 d of ripening compared to Model 4 (six genes) and Model 5 (three genes) (Table 7). Validation of Model 6 using the data collected from cold or ethylene conditioning treatments in 2012 and 2013 generated an R^2 of 0.638 (Table 7). We also examined the potential of using *ACS1a* in the model. However, this gene was excluded in further model validation due to its low expression and sometimes undetectable levels (data not shown). The existence of multiple isoforms of ACSs (El-Sharkawy et al., 2004; Villalobos-Acuna et al., 2011) may be the reason for the low expression of *ACS1a*.

Table 6: Pearson correlations between predictors in the multiple linear regression models using 2010 data.

	RE _{ACO}	RE _{ACS1a}	RE _{ETR1a}	RE _{ETR2}	RE _{ERS1a}	RE _{CTR1}	LogF
RE _{ACO}	1.00	0.84	0.61	0.76	0.83	-0.75	-0.93
RE _{ACS1a}	0.84	1.00	0.67	0.69	0.79	-0.64	-0.83
RE _{ETR1a}	0.61	0.67	1.00	0.66	0.70	-0.09	-0.59
RE _{ETR2}	0.76	0.69	0.66	1.00	0.93	-0.36	-0.73
RE _{ERS1a}	0.83	0.79	0.70	0.93	1.00	-0.43	-0.82
RE _{CTR1}	-0.75	-0.64	-0.09	-0.36	-0.43	1.00	0.77
LogF	-0.93	-0.83	-0.59	-0.73	-0.82	0.77	1.00

Table 7: Regression models to predict firmness after 6 d of ripening based on relative expression of ethylene genes (using 2010 data) after 0 d of ripening, and the validation (using 2012, 2013, and 2014 data).

	Model (2010)	RMSE	R ²	R ² adj	R ² Validation	
					2014	2012-13 [†]
4	$\text{LogF} = 8.999 - 0.665\text{RE}_{\text{ETR1a}} + 0.213\text{RE}_{\text{ETR2}} - 0.693\text{RE}_{\text{ERS1a}} + 1.514\text{RE}_{\text{CTR1}} - 0.061\text{RE}_{\text{ACO}} + 0.060\text{RE}_{\text{ACS1a}}$	0.117	0.928	0.914	0.499	NA
5	$\text{LogF} = 9.713 - 0.650\text{RE}_{\text{ETR1a}} - 0.550\text{RE}_{\text{ERS1a}} + 1.693\text{RE}_{\text{CTR1}}$	0.130	0.919	0.912	0.501	NA
6	$\text{LogF} = 3.412 - 0.308\text{RE}_{\text{ACO}}$	0.211	0.870	0.866	0.640	0.638

p-value of all models $\leq 2e-16$; RMSE: Root mean squared error; adj: adjusted; [†]: Validation was conducted on RE_{ACO}; NA: not applicable

Table 8: Coefficients of Model 4

Coefficients	Estimate	SE	p-value
(Intercept)	8.999	2.089	0.000
RE _{ETR1a}	-0.665	0.241	0.009
RE _{ETR2}	0.213	0.195	0.281
RE _{ERS1a}	-0.693	0.231	0.005
RE _{CTR1}	1.514	0.347	0.000
RE _{ACO}	-0.061	0.054	0.265
RE _{ACS1a}	0.060	0.048	0.221

4. Conclusions

In summary, the cold conditioning treatments generated a gradient of ripening capacity in ‘Bartlett’ pears. These fruit were then used to develop models to predict fruit firmness after 6 d of ripening at 20 °C based on expression of genes associated with ethylene biosynthesis and signaling that were formed in the fruit immediately after the cold conditioning and before fruit ripening. Through two methods of validation, it was shown that the ACO model is more robust. It is promising to use the RE of ACO in unripe ‘Bartlett’ pears to predict the ability of fruit to ripen within 6 d at 20 °C. A future approach would investigate the possibility of using protein levels of ACO in a rapid kit to predict ripening capacity and also to develop kits for additional European pear cultivars. These kits would provide a practical tool that will assist the pear industry to predict how specific fruit will behave during ripening so they can more efficiently deliver fruit with an appropriate ripeness stage to consumers.

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