Final Report

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Project Title: Using Chlorophyll Fluorescence for the Rapid, Non-Destructive Assessment of Apple Fruit Quality

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Overall objectives:

Chlorophyll fluorescence (CF) has been used to measure the physiological status of whole plants and plant organs, including apple fruit. In apples, CF has been proposed as an indicator of ripening and senescence, and physiological disorders caused by low O_2, high CO_2, heat, and freezing (DeELL, et al.1995, Song, et al., 2001, and Fan et a l., 2005). Also a close relationship between CF and firmness as well as ground color has been reported (Song, et al., 1997). Fluorescence measurements are rapid (0.3 to 0.8 s) and can be made on the surface or at some distance from the fruit surface, thus having potential use for in-line sorting. Identifying the relationships between CF and internal quality indices such as firmness, soluble solids, acidity and aroma, may lead to the development of a new sorting mechanism that will help the apple industry to ensure high eating quality of fruit. The objectives of this study were:

To characterize the relationship between chlorophyll fluorescence and the eating quality indices of firmness, sweetness, acidity, SS/TA and aroma production.
To determine the effects of cultivar, maturity, storage, and 1-MCP on this relationship.
To identify chlorophyll fluorescence parameters that can be used to sort fruit for internal quality.

Progress and significant findings (2002-2003):

1. Significant relationship between CF and the fruit quality parameters firmness, TA, SS/TA and aroma volatile content was found in ‘ Red Delicious’, ‘Jonagold’, ‘Fuji’ and ‘Gloster’ apples.
2. Among the CF parameters, Fm, Fv, Fv/Fm, Fv/Fo and Fo/Fv showed a significant relationship with the quality parameters firmness, TA, SS/TA and butyl acetate (BA) and 2-methylbutyl acetate (2MBA) content.
3. No significant relationship was found between CF and SS.
4. Among the tested cultivars, >Red Delicious=, >Jonagold= and >Gloster= had a strong relationship between CF and firmness, TA, SS/TA and aroma production. >Fuji= had weak relationships between CF and Firmness.

5. Treatment with 1-MCP did not alter the relationship between CF and most quality parameters in >Red Delicious=. The relationship between CF and firmness was lower in 1-MCP treated fruit in >Jonagold= but higher in >Fuji=.

Progress and significant findings (2003-2004):

1. Significant relationships between CF and fruit firmness, TA, SS/TA and aroma volatile content were found in 'Red Delicious' 'Gala' and Golden Delicious' apples. A similarly significant relationship was found in >Red Delicious= this year again, which confirmed last year=s results. A good relationship was also found in two additional cultivars >Gala= and >Golden Delicious=.
2. Among the CF parameters, Fv, Fm, Fv/Fm, and Fv/Fo showed significant relationships with the quality indices, firmness, TA, SS/TA and butyl acetate (BA) and 2-methylbutyl acetate (2MBA) content. Fo and Fo/Fv were found not to be good indicator for quality prediction.
3. No significant relationship was found between CF and SS.
4. Treatment with 1-MCP decreased the strength of the correlation between CF and most quality indices in all three cultivars. However, 1-MCP treatment did not weaken the relationship between Fv (one of the CF parameters) and firmness in >Red Delicious=. Treatment with 1-MCP also did not change the relationship between CF and BA and 2-MBA production in all cultivars.
5. The correlation between CF and quality of >Red Delicious=, >Jonagold= and >Fuji= fruit following CA storage (3, 6 and 9 months) were weak compared with the ripening study due to little changes in quality indices between day 1 and day 7 after CA storage.
6. Fruit temperature and light conditions during measurement will affect CF measurements when Fo, Fv and Fm are used. However, there is no effect when Fv/Fm or Fv/Fo was used.

Progress and significant findings (2004-2005):

1. Mathematical models were established between CF measurement and eating quality parameters in "Red Delicious" apple. Fv was identified as the most constant CF parameter for fruit quality measurement.
2. Data analysis of the relationship between CF and fruit quality indices after CA storage (3, 6 and 9 months) was completed.
3. The experiment to investigate the correlation between CF and quality of >Red Delicious= and >Golden Delicious= fruit after CA storage was redesigned. Fruit harvested from three different maturities are beign evaluated following CA storage (3, 6 and 9 months) and the last sampling will be finished by the end of July.

Results and Discussion (2004-2005)

Mathematical models of CF and fruit quality.

Evaluations of the relationship between CF and fruit quality indices have been conducted on 'Jonagold', 'Fuji', >Red Delicious= , 'Gala' , 'Gloster' and Golden Delicious'. Among the tested cultivars, 'Red Delicious' was repeated for three years. Mathematical models between CF and fruit quality indices for 'Red Delicious' have been established after data analysis from multi-years. The formula $X=\ln^{((Y-A)/B)}/\ln R$ can be used to illustrate the non-linear relationship between CF and fruit quality indices. Among all CF parameters, Fv was identified as the most constant CF parameter, although Fv/Fo, Fv/Fm and Fm also show significant relationships. Significant relationships between CF and firmness and SS/TA ratio in control and 1-MCP treated fruit were modeled (Fig. 1A, 1B, 1C, 1D, 1E and 1F). Significant relationships between CF and production of butyl acetate and 2-mehtylbutyl acetate were also found and modeled (Fig.2A and 2B). 1-MCP treatment did not influence the relationship between CF and firmness, decreased slightly the relationship between CF and titritable acidity (TA) and SS/TA, but increased significantly the relationship between CF and volatile production with a R^2 value of 0.85.

Relationship between CF and fruit quality indices after CA storage (3, 6 and 9 months):

In the previous year, >Red Delicious=, >Gala' and 'Golden Delicious' were stored under CA conditions for 3, 6 and 9 months. After CA storage, fruit were held at 20 C and evaluated after 1 or 7 days. Two separate statistical analyses were conducted to explore the relationship between CF and fruit quality indices. First, all day 1 and day 7 data were analyzed over the entire storage period, and second, the day 1 and day 7 data were combined and the 3, 6, 9 months evaluations were analyzed separately. Under these conditions, relatively weak relationships were found in all three cultivars. The relationship between CF and firmness, TA and SS/TA were all low with R^2 value around 20-30%. However, volatile production in >Gala" 'Red Delicious' and 'Golden Delicious' were significantly higher with R^2 value from 40 to 60%. Comparing the data with apples during normal ripening, it is observed that the changes in quality indices such as firmness, TA and SS/TA were too little during the observing period. The small range of change in quality indices such as firmness and SS/TA ratio may be responsible for this weak relationship. In 2004-2005, we have redesigned the approach to study CA effects on the relationship between CF and fruit quality by harvesting apples ('Red Delicious' and 'Golden Delicious') at three different ripening stages from early harvest to late harvest. Evaluations were conducted at 3, 6 and 9 months at day 1 and day 7 after CA storage. Data will be presented later.

Commercialization of CF technology

Discussions between our group and Satlantics, inc. Halifax, Nova Scotia were conducted, but no progress has been made to develop prototype sensors.

Results and discussions (general)

Overall relationship. The relationship (R^2) between CF and all quality indices during fruit ripening are illustrated in Table. 1. Among the CF parameters, Fv, Fm, Fv/Fm, Fv/Fo are good parameters relating to fruit quality indices. Results have been tested on 'Jonagold', 'Fuji', 'Gloster', >Red Delicious=, 'Gala' and 'Golden Delicious'. Repeated results have been obtained from >Red Delicious= fruit indicating that CF measurements are repeatable and reliable. Good relationships were also found in 'Jonagold', >Gala= and >Golden Delicious=. In our study, Fv/Fo was also found to be good indicator for fruit quality measurement. In this study, the weak relationship between Fo and quality indices indicates that Fo is not suitable for prediction of fruit quality during ripening of apple fruit. In general, the parameters of CF measurement are somehow related, among them Fv is the best parameter to be used for quality detection.

CF and firmness. Relationship between CF and firmness of fruit was different among cultivars. In 'Red Delicious', a strong relationship between Fv, Fm, Fv/Fm and Fv/Fo and firmness was found with R^2 values of 77-78%, 70-74%, 78-79% and 81-81%, respectively (Table.1 and 3). In 'Gala=, similar relationships were found being around 69%, 63%, 73% and 74%. In 'Golden Delicious= the relationship between Fv, Fm and firmness was the same as >Gala=, but lower in Fv/Fm and Fv/Fo, being around 33% and 32%. Similar relationship was also found in 'Jonagold' and 'Gloster' with R^2 value between 60-70%. There is evidence that the relationship between CF and fruit firmness is physiologically dependent and consistent in >Red Delicious=. Using CF to measure fruit firmness for 'Red Delicious', 'Gala= 'Jonagold' and >Golden Delicious= is promising.

CF and TA, SS/TA and SS. A good relationship between CF and TA and SS/TA was also found. Overall, the R^2 values ranged from 60% to 76%, when Fv, Fm, Fv/Fm and Fv/Fo were measured. The relationship between CF and SS/TA in 'Red Delicious' is slightly better in 2002 than in 2003 with R^2 value of 60 % up to 76%, 76%, 60% and 61% (Table.1 and 3). The R^2 values for TA and SS/TA and Fv and Fm ranged from 50% to 70% in 'Gala= and >Golden Delicious=. In 'Jonagold', good relationship between CF and TA and SS/TA was also found. During fruit ripening and senescence, TA decreased

while SS/TA ratio increased, which correlated with a decrease in CF. It is promising that this relationship could be useful to measure fruit eating quality using CF technology.

No relationship was found between CF and SS due to the variability among the individual fruit (data not shown), which has been confirmed by three year findings from all cultivars. This indicates fruit SS were cultivar and harvest dependent and changed little during fruit ripening.

CF and volatile production. During this study, more than 20 aroma compounds were identified. However, butyl acetate and 2-methylbutyl acetate have been analyzed. These two volatile represent two different aroma volatile biosynthesis pathways in apples. A strong relationship between CF and volatile production was found in>Gala= and >Golden Delicious=, R^2 values of the aroma volatiles butyl acetate and 2-methyl butyl acetate were about 62 % and 74% in 'Gala' and 'Golden Delicious', when Fv and Fm were measured.

Effect of 1-MCP treatment on CF and quality indices. The relationships between CF and quality indices were affected by 1-MCP treatment. However the effects varied with different cultivars and CF parameters. It was found that there was no difference in the relationship between CF and firmness in control and 1-MCP treated >Red Delicious= fruit when Fv was measured (Table 1, 2 3,and 4). In all other cultivars, however, the relationship between CF and firmness, TA and SS/TA were lower ranging from 40% to 60% (Table 2 and 4). 1-MCP treatment strongly affected the decrease in TA. However, strong relationship between CF and SS/TA was found in 'Fuji' with 1-MCP treatment. On the other hand, significant relationship has always been found between CF and volatile production in 'Gala' and 'Red Delicious' with 1-MCP treatment.

Factors influencing CF measurement.

Two important factors that may influence CF measurements under commercial conditions were evaluated, fruit temperature (cold and warm) and light. The data were collected from >Red Delicious= fruit after 5 months of CA or air storage. At measurement, fruit temperatures were 10 C or 20 C for two groups of fruit. Light intensity was 8.5 $\mu mol^{-1}\ s^{-1}\ m^{-2}$ PAR with the light on in the lab and 0.15 $\mu mol^{-1}\ s^{-1}\ m^{-2}$ PAR in the same darkened lab with the light out. Statistical analysis (ANOVA) indicated that Fo, Fv and Fm measurement alone were influenced by fruit temperature. The warm fruits were found to have higher Fo, Fv and Fm values. Light conditions affected only Fo; with light the Fo value increased. It is also interesting that the increased value of Fo, Fv and Fm are proportionally similar, therefore, there is no difference in CF measurement when ratios are used, such as Fv/Fm and Fv/Fo. This finding indicated that CF is related to physiological activity of chlorophyll, which is dependent upon many environmental conditions such as temperature and light. Fruit temperature and light should not influence the outcome of chlorophyll fluorescence when Fv/Fm and/or Fv/Fo are used. Under circumstances, however, when Fv and Fm are to be used, it would be necessary to keep measurement conditions consistent, especially fruit temperature.

Previous studies reported that Fv/Fm and Fm were good indicators for fruit firmness measurement in 'Red Delicious' and 'Law Rome'. Both Fo and Fm can be used to predict the change of ground color of
'Golden Delicious' fruit (Song, et al., 1997). In our study, Fv/Fm, Fv/Fo, Fv and Fm were all found to be good indicators of fruit quality in apples. ANOVA indicated that Fo and Fo/Fv are not as good as Fv/Fm, Fm, Fv and Fv/Fo in apples.

Potential applications

Eating quality of apples is very complex combination of texture and flavor components. Non-invasive detection of eating quality of apples has been a challenge for both fruit industry and research. Our study proved that CF is a physiological parameter that can provide a meaningful nondestructive tool to monitor fruit quality. While CF technology does not measure quality indices directly, it measures the physiological statues of fruit, which closely relates to fruit quality. It is possible to use different CF parameters such as Fv, Fm, Fv/Fm or Fv/Fo to predict fruit quality indices, or to establish a threshold or cut-off value to segregate fruit quality/market life into different groups. The relationships between CF and fruit quality indices that have been found so far are based on a pulse modulated fluorometer (Opti-Sicence) with a small sensor (~0.65 cm^2) and repeated measurements. It is possible that improved CF technology together with further analysis of data and mathematical modeling could improve the accuracy and reduce errors of measurement, which will further define the relationship between CF and firmness, TA and SS/TA. It is promising that this information may lead to the development of a non-destructive fruit quality detector to sort fruit based on eating quality prior to fruit being shipped to the market/consumers.

References

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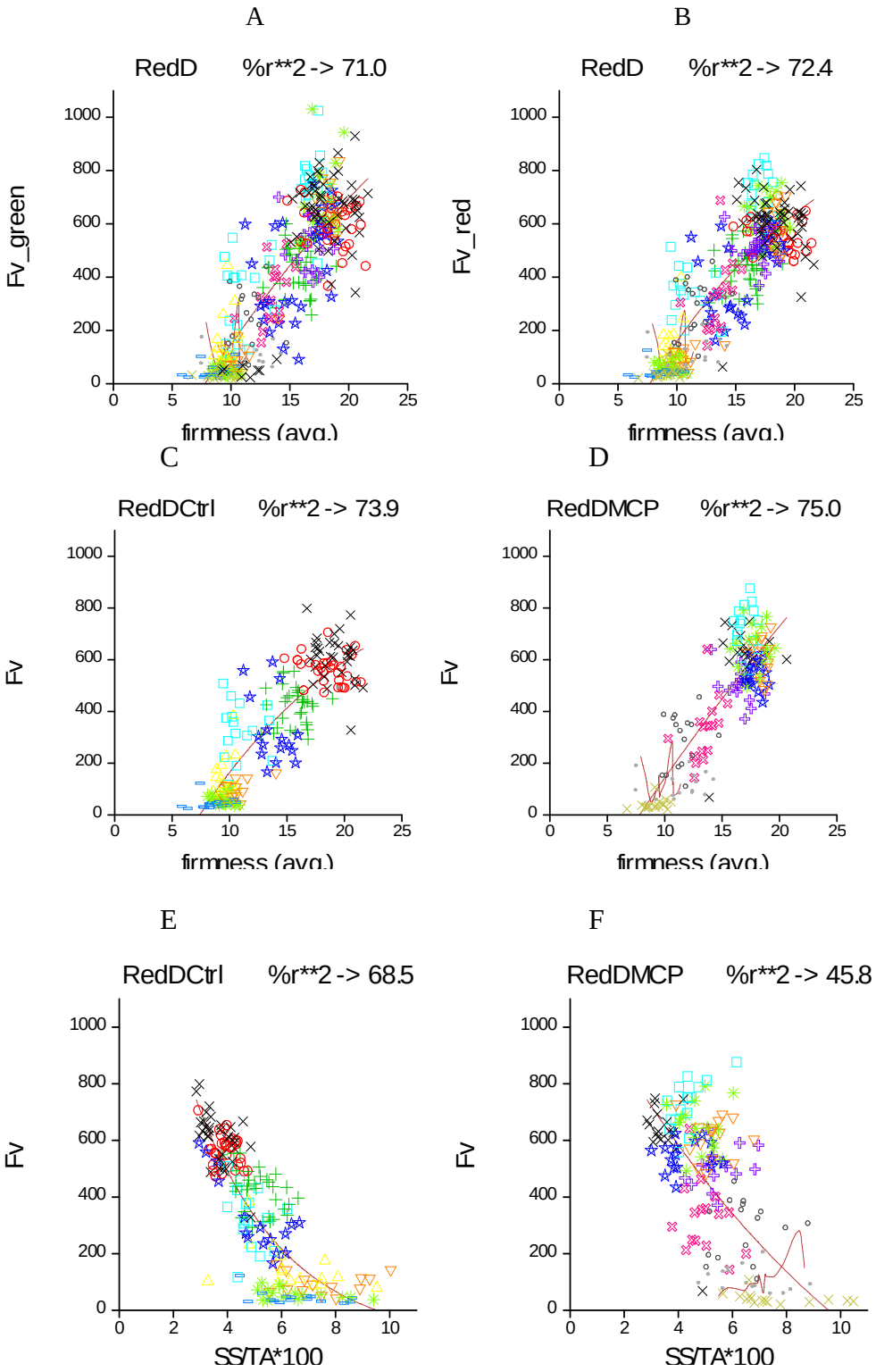

Fig. 1. The relationship between chlorophyll fluorescence (Fv) and firmness and Soluble solids/titratable acidity (SS/TAx100) in 'Red Delicious' apple fruit during ripening. Regression

curve is illustrated by the model: $X=\ln^{((Y-A)/B)}/\ln R$. Points represent individual fruit following ripening at 20 °C for up to 38-42 days. A: measurement on green side; B; measurement on red side; C: control; D: 1-MCP; E: CF and SS/TA control; F: CF and SS/TA 1-MCP.

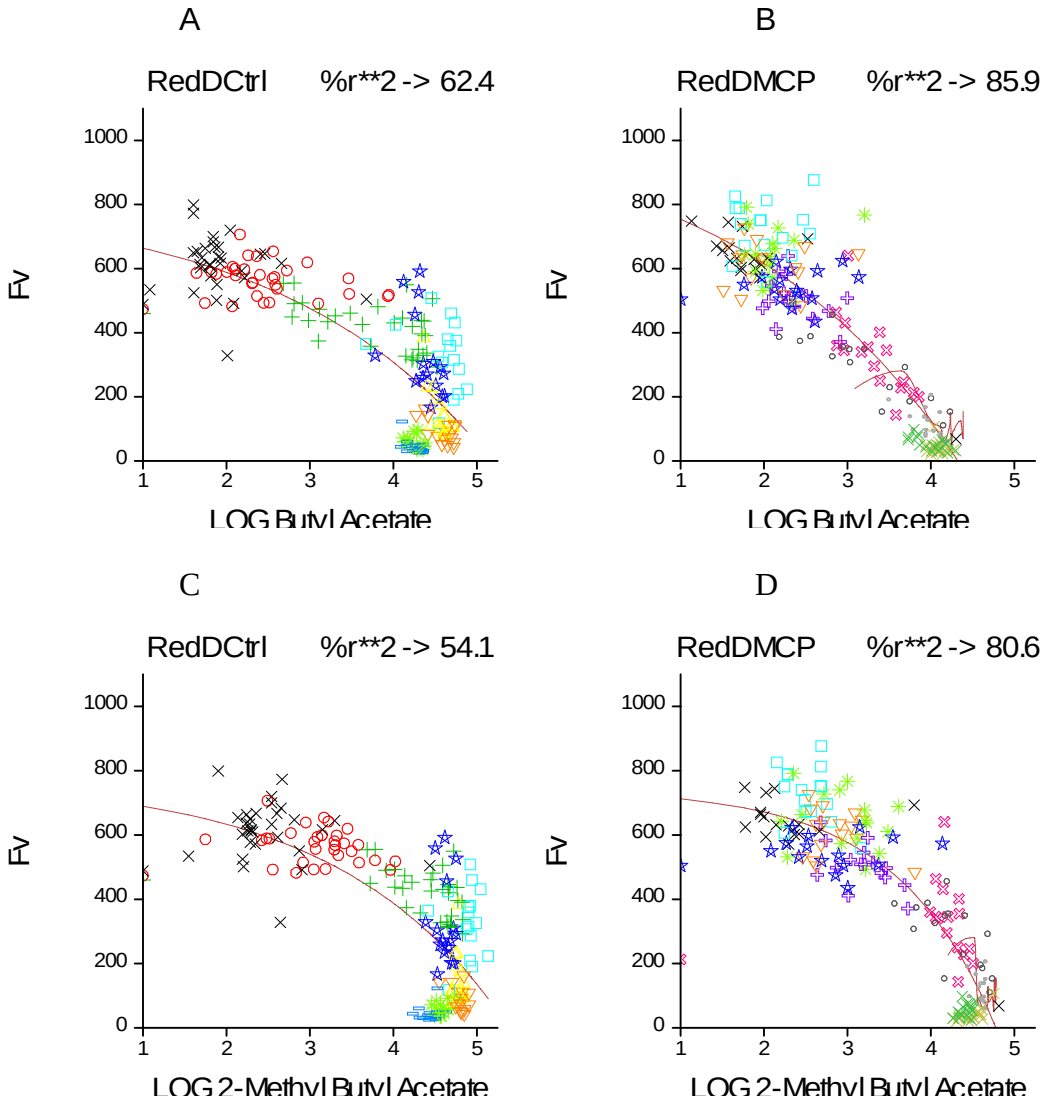

Fig. 2. The relationship between chlorophyll fluorescence (Fv) and production of volatile aroma compounds butyl acetate (BA) and 2-methyl butyl acetate (2-MBA) in 'Red Delicious' apple fruit during ripening. Points represent individual fruit following ripening at 20 °C for up to 38-42 days. A: CF and butyl acetate control; B: CF and butyl acetate, 1-MCP; C: CF and 2-methyl butyl acetate; D: CF and 2-methyl acetate 1-MCP.

Table 1. Relationship (R^2) between CF parameters and fruit quality measurements during fruit ripening at 20C (2002-2003)

	>Jonagold=			>Red Delicious=			>Gloster=			>Fuji=		
	Firmness	TA	SS/TA	Firmness	TA	SS/TA	Firmness	TA	SS/TA	Firmness	TA	SS/TA
Fo	26	25	28	16	27	26	41	.41	45	25	26	24
Fv	60	57	60	77	55	63	32	15	18	4	38	41
Fm	57	54	58	70	55	62	10	9	16	6	15	30
Fv/Fm	52	58	55	79	53	52	62	38	54	3	50	50
Fo/Fv	37	53	49	60	45	39	34	34	56	4	36	30
Fv/Fo	59	55	55	83	49	57	64	49	67	15	50	53

Table 2. Relationship (R^2) between CF parameters and fruit quality measurements in 1-MCP treated fruit during fruit ripening at 20C (2002-2003).

	>Jonagold=			>Red Delicious=			>Gloster=			>Fuji=		
	Firmness	TA	SS/TA	Firmness	TA	SS/TA	Firmness	TA	SS/TA	Firmness	TA	SS/TA
Fo	9.2	10	7	21	24	21	8	7	13	9	29	31
Fv	35	42	52	80	61	55	26	20	25	27	55	67
Fm	32	39	44	71	56	44	37	45	52	12	18	30
Fv/Fm	30	38	50	69	61	67	7	3	6	32	65	82
Fo/Fv	21	25	32	41	43	66	9	7	17	11	13	32
Fv/Fo	26	37	50	61	66	38	29	50	64	20	64	77

Table 3. Relationship of R^2 (%) between CF parameters and fruit quality measurements during fruit ripening at 20 C (2003-2004).

	'Red Delicious'			'Gala'			'Golden Delicious'		
	Firmness	TA	SS/TA	Firmness	TA	SS/TA	Firmness	TA	SS/TA
Fo	39	48	47	20	17	19	56	51	61
Fv	78	71	76	69	54	62	68	57	66
Fm	74	73	76	63	51	57	68	57	67
Fv/Fm	78	60	60	73	71	71	33	19	20
Fo/Fv	69	54	52	47	52	49	29	17	18
Fv/Fo	81	57	61	74	62	68	32	19	19

Table 4. Relationship R^2 (%) between CF parameters and fruit quality measurements in 1-MCP treated fruit during fruit ripening at 20C (2003-2004).

	'Red Delicious'			'Gala'			'Golden Delicious'		
	Firmness	TA	SS/TA	Firmness	TA	SS/TA	Firmness	TA	SS/TA
Fo	35	6	8	24	8	14	44	35	31
Fv	75	22	19	47	36	39	52	46	55
Fm	54	9	15	46	33	33	58	50	54
Fv/Fm	65	39	38	50	52	61	17	10	14
Fo/Fv	45	25	22	49	49	49	28	17	13
Fv/Fo	59	35	38	31	39	60	14	13	15

Table 5. Relationship (R^2) between CF parameters and fruit aroma production (BA and 2-MBA) during fruit ripening at 20C.

	'Red Delicious'		'Gala'		'Golden Delicious'	
	BA	2-MBA	BA	2-MBA	BA	2-MBA
Fo	25	32	18	15	63	65
Fv	61	67	61	62	72	74
Fm	51	66	55	56	72	74
Fv/Fm	38	53	45	45	30	31
Fo/Fv	26	34	22	37	29	28
Fv/Fo	51	56	64	61	27	27

Table 6. Relationship R^2 (%) between CF parameters and fruit aroma production in 1-MCP treated fruit during fruit ripening at 20C (2003-2004).

	'Red Delicious'		'Gala'		'Golden Delicious'	
	BA	2-MBA	BA	2-MBA	BA	2-MBA
Fo	5	12	18	24	18	16
Fv	51	84	61	33	24	19
Fm	41	68	55	34	20	24
Fv/Fm	58	64	45	15	18	16
Fo/Fv	46	37	22	8	17	15
Fv/Fo	61	61	64	15	17	14