Chilling injury in stored nectarines and its detection by time-resolved reflectance spectroscopy

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ABSTRACT

Nectarine fruit after cold storage soften normally, but become dry instead of juicy and can develop flesh browning, bleeding and a gel-like or glassy formation of the flesh near the pit. An experiment was conducted to see if time-resolved reflectance spectroscopy could distinguish these internal disorders non-destructively. The optical parameters of absorption coefficient (μ_a) and reduced scattering coefficient (μ'_s) were measured at 670 nm and 780 nm, on nectarine (*Prunus persica* cv. Morsiani 90) fruit held at 20 °C after harvest or after 30 d of storage at 0 °C or 4 °C. Each day for 5 d 30 fruit were examined both non-destructively and destructively. Other measurements were firmness with a penetrometer, peel colour on the blush and non-blush side, expressible juice, weight loss, and visual rating of internal browning, bleeding, and gel. The fruit had been sorted at harvest according to the value of μ_a670 so that each batch had a similar spread of fruit maturity. More mature fruit (lower μ_a670 values) developed internal browning and bleeding with more severe symptoms compared to less mature ones (higher μ_a670 values). It was found that μ_a780 could distinguish healthy fruits from the chilling injured ones. Canonical discriminant analysis indicated that fruit without cold storage had low μ_a780 , less water loss, low firmness, but high μ_a670 and high expressible juice compared with cool stored fruit. Fruit cool stored at 4 °C had high μ_a780 and less expressible juice, lower water loss and lower firmness compared with fruit cool stored at 0 °C. It was concluded that time resolved reflectance spectroscopy could detect internal woolliness and internal browning in nectarines after storage.

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1. Introduction

Peaches and nectarines ripen and deteriorate quickly at ambient temperature. Cold storage is used to slow these processes and decay development. However, low temperature disorders, chilling injury (CI) classified as internal breakdown, limit the storage life of peaches and nectarines under refrigeration. The onset of chilling injury symptoms determines the postharvest storage/shipping potential because their development reduces consumer acceptance. CI is genetically influenced and triggered by a combination of storage temperature and storage period (Crisosto et al., 1999; Peace et al., 2004). It manifests itself as fruit that are dry and have

a mealy or woolly texture (woolliness), or hard textured fruit with no juice (leatherness), fruit with flesh browning (internal browning), or with flesh bleeding (internal reddening) (Lurie and Crisosto, 2005).

It has been widely reported that the expression of CI symptoms, especially flesh browning and woolliness, develops faster and more intensely when susceptible fruit are stored at temperatures between 2.2 and 7.6 °C (killing temperature zone) than those stored at 0 °C (Harding and Haller, 1934; Crisosto et al., 1999). These symptoms mainly develop during fruit ripening after cold storage, and the problem is not noticed until the fruit reaches customers (Bruhn et al., 1991; Crisosto et al., 1995). Since the problem develops following storage, it would be advantageous if there was a way to determine CI, so as to remove the affected fruit from the marketing chain and increase consumer satisfaction, and return purchases.

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Time-resolved reflectance spectroscopy (TRS) is a nondestructive technique based on the measurement of the temporal delay and broadening experienced by a short laser pulse (pulse duration in the order of 100 ps) while travelling through a turbid medium, such as fruit flesh (Torricelli et al., 2008). By using an appropriate theoretical model of light penetration for the analysis of photon time distribution, it is possible to simultaneously estimate the absorption coefficient (μ_a) and the reduced scattering coefficient (μ'_s). The absorption and reduced scattering coefficients correspond, respectively, to the probability (per unit distance travelled by a photon) of being absorbed by the main fruit components (water, chlorophyll, carotenoids and sugars) and of changing direction due to microscopic changes in refractive index caused by membranes, air, vacuoles, or organelles. TRS probes fruit and vegetable to a depth as great as 1–2 cm, depending on the optical properties (Cubeddu et al., 2001).

It was found that the absorption coefficient at 670 nm (μ_a670), the absorption peak of chlorophyll-a, is linked to fruit biological age, and decreases as fruit ripen (Tijssens et al., 2006). In nectarines the conversion of μ_a670 to a biological shift factor was successfully used to predict fruit softening rate during shelf life (Eccher Zerbini et al., 2006; Tijssens et al., 2007), allowing the selection of fruit for different market destinations (Eccher Zerbini et al., 2009). By using μ_a670 , it was possible to select nectarines having distinctive sugar, acid and aroma compositions, as well as sensory characteristics (Jacob et al., 2006; Bianchi et al., 2004; Eccher Zerbini et al., 2003). Furthermore, in apples a relationship was observed between optical properties, mainly the scattering coefficient, and the 'mealy' sensory attribute. In fact, mealiness increased with increasing scattering coefficient measured at 780 nm (μ'_s780) in 'Jonagored' apples (Vanoli et al., 2007), and increasing scattering coefficient measured at 790 nm and 912 nm (μ'_s790 and μ'_s912) and decreasing absorption coefficient measured at 912 nm (μ_a912) in 'Braeburn' apples (Vanoli et al., 2010). In addition, in Granny Smith apples the absorption coefficient measured at 750 nm (μ_a750) was higher in fruit having internal browning compared to the healthy fruit (Vanoli et al., 2010).

In the present study, the optical parameters of absorption coefficient (μ_a) and reduced scattering coefficient (μ'_s) measured at 670 nm and 780 nm, by TRS were used to detect chilling injuries in stored nectarines.

2. Materials and methods

2.1. Plant material and storage

Nectarines (*Prunus persica* cv. Marsiani 90) were harvested on 13 September 2009 in a commercial orchard in Faenza (Italy) and 480 fruit without defects and bruises were selected. At harvest, each fruit was measured on two sides by TRS using a prototype built at Politecnico di Milano (Torricelli et al., 2008) and ranked by decreasing absorption coefficient at 670 nm (μ_a670) averaged over the two fruit sides, i.e. from less mature to more mature fruit (Eccher Zerbini et al. 2006; Tijssens et al., 2007). Ranked fruit were grouped into 16 sets of 30 fruit, corresponding to 30 levels of μ_a (10 less mature class, 10 medium mature class and 10 more mature class). Then each fruit within each set was randomly assigned to a different group (i.e. time of analysis) in order to have fruit from the whole range of μ_a at every time of analysis. One group was analyzed at harvest, while the other 15 groups were assigned for analysis during shelf-life at 20 °C. There were three treatments, each with 5 times of analysis (groups): five groups were put in shelf life at 20 °C (RH ≈ 78%) at harvest without any storage and analyzed after 1, 2, 3, 6 and 7 d; five groups were stored at 4 °C (RH ≈ 90%) for 4 weeks, and then put in shelf life at 20 °C and analyzed at day 1, 2, 3, 4 and

5; the other 5 groups were placed into 0 °C (RH ≈ 70%) storage for 4 weeks and then analyzed during shelf life at 20 °C after 1, 2, 3, 4 and 5 d. During shelf life fruit were kept in a controlled temperature room at 20 °C.

2.2. Measurements

At harvest all the fruit were individually measured for the following parameters: absorption and reduced scattering coefficients at 670 and 780 nm (μ_a670 , μ_a780 , μ'_s670 and μ'_s780), weight, peel colour on the blush and opposite side at two scales, L^* , a^* , b^* and L^* , C^* , H° using a spectrophotometer CM-2600d (Minolta Co., Japan). These parameters were measured again at each time of analysis during shelf life. Weight loss was calculated from the initial and final weight. Firmness on blush and non-blush side was determined with an 8 mm diameter plunger mounted on a Texture Analyzer TA.Xtplus (Stable Micro Systems, England), with crosshead speed of 200 mm/min, on the two peeled cheeks of each fruit, in positions corresponding to the two TRS readings. A plug was removed from each fruit and a 1 cm section just below the peel was taken for determination of expressible juice by the method of Lill and van der Mespel (1988). Each fruit was then halved and examined visually for chilling injuries (gel formation, internal browning and internal reddening). The disorders were rated on a scale of 1–3, with 1 healthy and 3 when all the flesh was affected. For each disorder a severity index was computed by multiplying the number of fruit in each score class by the score of the class and dividing their sum by the maximum possible score (as if all fruit had score 3). In addition, within the same sample (time of analysis), the fruits belonging to the same TRS maturity class (less mature, medium mature and more mature) were calculated for the chilling injuries. Furthermore stored fruit were separated according to pulp defects into the following seven categories, regardless of storage temperature and day of shelf-life; healthy (H), only browning (BR), only gel (G), only bleeding (BL), browning associated to gel (BR + G), bleeding associated to browning (BL + BR) and bleeding associated to both browning and gel (BL + BR + G).

2.3. Statistics

Firmness, weight loss, expressible juice, optical properties and colour parameters were submitted to analysis of variance (ANOVA) considering as factors the day of shelf life and the treatment (GLM procedure, SAS Version 9.1, SAS Institute Inc., USA). Means were compared by Tukey's test at $p < 0.05\%$.

In order to study the relationships among the optical properties and the presence of bleeding, browning and gel, the data variables of the optical properties measured in shelf life after storage at 0 °C and 4 °C (μ_a670 , μ_a780 , μ'_s670 and μ'_s780) and the scores of each fruit regarding bleeding, browning and gel were submitted to principal component analysis (PCA, Statgraphics, version 7, Manugistic, Inc., Rockville, MD). The PC1 and PC2 scores were then submitted to analysis of variance (ANOVA) and means were compared by 95.0 percent Bonferroni method considering as factors the categories of pulp defects. Correlation analysis between μ_a670 , μ_a780 , μ'_s670 and μ'_s780 and expressible juice was carried out considering separately the data of each treatment ($n = 150$).

Stepwise canonical discriminant analysis was used to select fruit attributes that made a significant contribution to discrimination of fruit into different treatments. In this case, only data from the last two days of shelf life (fruit without storage assessed on shelf days 6 and 7, 0 °C and 4 °C fruit assessed after 4 and 5 d shelf life) were used. There were 17 input variables (μ_a670 and μ_a780 , μ'_s670 and μ'_s780 , weight loss, L^* , a^* , b^* , C^* , hue for both background and blush side of the fruit, firmness and expressible juice) used for stepwise canonical discriminant analysis and only seven selected variables

Fig. 1. Firmness of nectarines held at 20°C without storage, or following 30 d storage at 4°C or 0°C. Standard error is indicated.

Fig. 2. Weight loss of nectarines held at 20°C without storage, or following 30 d storage at 4°C or 0°C. Standard error is indicated.

were used for Canonical discriminant analysis (Cruz-Castillo et al., 1994). Canonical discriminant analysis was undertaken using the software package SAS Version 9.1 (SAS Institute Inc., USA).

3. Results

3.1. Fruit softening and peel colour

The fruits lost firmness during the shelf life period at 20°C (Fig. 1). Fruits stored at 0°C were of the same firmness as at harvest when removed from storage, but softened over the 5 d period at 20°C to a similar extent as non-stored fruit. During 4°C storage there was 40% loss of firmness (from 52 ± 12 N to 30 ± 7 N) and the fruit advanced in ripeness, but continued to soften once removed. The trend of fruit softening was similar in all three treatments, although the final firmness of fruit from 4°C storage was lower than that of fruit from 0°C storage after 5 d, and similar to that of non-stored fruit on day 7.

Weight loss of the nectarine fruit during 20°C shelf life is shown in Fig. 2. The least weight loss during shelf life was found in non stored fruit, and the highest in fruit stored at 0°C, due to the lower relative humidity in that storage room than in the 4°C room.

Peel colour changes are shown in Table 1 for the three treatments. On the non-blush side the L^* (lightness) value was higher, indicating a lighter peel, for fruits from 4°C storage than the other

treatments. The H° value (hue angle) decreased during shelf life in all treatments, indicating a yellowing of the peel, while the C^* (chroma) values increased. On the blush side all the measurement had large standard errors, probably because of great variation in the intensity of blush on the fruit. There were no large differences among the treatments, and none that were significant because of the great variation.

3.2. Expressible juice

Woolliness in nectarines, as a result of cold treatment, was measured by determining expressible juice. In expressible juice, the less juice that can be extracted, the greater the woolliness. The changes in expressible juice were quite different for fruit that were not stored or stored (Fig. 3). For fruit after cold storage (0 and 4°C) a decrease was observed during shelf life, while for the non-stored fruit, an increase in expressible juice occurred as they decreased in firmness. For the first 3 d of shelf life, non-stored fruit had expressible juice of 45–50%, while for the last two days of shelf life, this increased to close to 60%. The stored fruit from both temperatures of storage began with 50% of expressible juice on the first day, and this decreased steadily to below 30% after 5 d.

Table 1
Peel color (L^* , C^* , H°) of nectarine fruit during shelf life at 20°C. Standard error is indicated.

Days at 20°C	Non-stored fruit			0°C			4°C storage		
	L^*	C^*	H°	L^*	C^*	H°	L^*	C^*	H°
Non-blush side									
1	75.1 ± 0.4	53.8 ± 0.4	86.3 ± 0.4	75.8 ± 0.3	52.9 ± 0.3	85.5 ± 0.6	78.5 ± 0.3	54.5 ± 0.4	84.0 ± 0.3
2	74.0 ± 0.3	53.7 ± 0.4	85.6 ± 0.6	76.2 ± 0.4	55.2 ± 0.4	82.6 ± 0.5	77.8 ± 0.2	54.7 ± 0.4	82.7 ± 0.3
3	74.7 ± 0.5	54.7 ± 0.5	84.2 ± 0.6	76.6 ± 0.3	54.7 ± 0.4	81.2 ± 0.4	78.1 ± 0.3	55.0 ± 0.4	81.9 ± 0.3
4				76.1 ± 0.4	55.6 ± 0.4	80.4 ± 0.5	76.4 ± 0.5	56.0 ± 0.6	80.0 ± 0.7
5				74.9 ± 0.5	56.7 ± 0.3	79.6 ± 0.5	76.0 ± 0.4	56.8 ± 0.3	80.0 ± 0.4
6	76.4 ± 0.2	57.0 ± 0.4	82.1 ± 0.4						
7	75.8 ± 0.3	57.4 ± 0.4	81.3 ± 0.3						
Blush side									
1	42.8 ± 1.5	40.1 ± 1.0	35.5 ± 2.2	46.4 ± 2.0	39.9 ± 1.4	39.4 ± 2.7	47.5 ± 1.7	41.9 ± 1.1	38.8 ± 2.1
2	44.0 ± 1.4	42.4 ± 0.7	37.1 ± 1.9	43.9 ± 1.9	41.0 ± 1.2	35.4 ± 2.4	43.7 ± 1.5	41.0 ± 1.0	33.7 ± 1.8
3	44.2 ± 1.6	42.8 ± 0.9	37.0 ± 2.1	44.2 ± 1.7	41.9 ± 1.2	35.8 ± 2.2	45.5 ± 1.8	42.8 ± 1.2	35.7 ± 2.1
4				43.8 ± 1.8	41.8 ± 1.2	35.4 ± 2.4	43.1 ± 1.8	40.4 ± 1.4	33.3 ± 2.3
5				42.2 ± 1.7	40.6 ± 1.3	33.7 ± 2.0	42.5 ± 1.1	41.1 ± 1.2	32.9 ± 1.5
6	43.1 ± 1.5	43.8 ± 1.1	34.0 ± 1.8						
7	44.4 ± 1.6	44.4 ± 1.1	35.8 ± 1.9

Fig. 3. Expressible juice of nectarines held at 20 °C without storage, or following 30 d storage at 4 °C or 0 °C. Standard error is indicated.

3.3. Internal disorders of browning, bleeding and gel

The development of changes in the fruit flesh was followed each day. During ripening without storage no disorders appeared (data not shown). However, fruit from both storage regimes had flesh disorders from the first day after storage, which increased continually during shelf life in fruit from 4 °C so that after 4 d there were no fruit with completely healthy flesh (Fig. 4). In fruit from 0 °C storage the increase in percentage of flesh disorders began after day 3 of shelf life, and by the end of shelf life was about 75% of the fruit. The time of appearance of the three flesh disorders, internal browning, internal bleeding and gel formation differed (Fig. 5). Gel formation and internal browning appeared on the second day of shelf life after 4 °C storage, and gel formation did not increase thereafter, while the internal browning became more intense on the last two days of shelf life. In contrast, bleeding was present immediately after removal from storage, and increased during the last two days at 20 °C. Fruit ripened after storage at 0 °C did not develop any gel, while internal browning and bleeding increased mainly on the last two days of shelf life.

Fruit which had been classified at harvest using μ_a670 as the 10 least and the 10 most mature fruit were examined as two groups for flesh disorders considering the severity indices. Internal browning appeared only in the more mature fruit from 0 °C, and was higher in more mature fruit from 4 °C than the less mature fruit (Fig. 5A). Gel formation was found only in fruit from 4 °C storage, and was higher

Fig. 4. The percentage of healthy fruit during shelf life at 20 °C of fruits held for 30 d at 4 °C or 0 °C.

Fig. 5. Browning (A), gel (B), and bleeding (C) severity indices of the 10 most mature (more) and 10 least (less) mature fruit held for 5 d at 20 °C after storage at 4 °C or 0 °C. Severity index = 0.3, all fruit without the defect (all scored 1), severity index = 1, all fruit having the defect scored 3.

at the beginning in the less mature fruit, but similar at the end of shelf life in both maturity groups (Fig. 5B). The bleeding severity index was similar in fruit from both storage temperatures, but in both cases was higher in the more mature fruit (Fig. 5C).

3.4. Changes in μ_a670 nm and μ_a780 nm

When the whole data set for the 5 d of each treatment was examined for the TRS measurements, it was found that the scattering coefficient μ'_s670 at harvest (mean $\pm$ standard error, $18.12 \pm 0.19 \text{ cm}^{-1}$) was significantly lower than values after cold

Fig. 6. Absorption coefficient at 670 and 780 nm of nectarines held at 20 °C without storage (at harvest, diamond), or following 30 d storage at 4 °C (triangle) or 0 °C (square). Diamond, fruit after harvest; square, fruit from 0 °C storage; triangle, fruit from 4 °C storage. Standard error is indicated.

storage (19.39 ± 0.21 cm⁻¹), whereas $\mu'_s 780$ did not significantly differ among treatments (17.63 ± 0.16 cm⁻¹) (data not shown). However the absorption coefficient at both wavelengths did differentiate among the treatments: the fruit that were stored at 4 °C had higher $\mu_a 670$ values (0.079 ± 0.002 cm⁻¹) than fruit without storage (0.067 ± 0.001 cm⁻¹) or after 0 °C storage (0.066 ± 0.001 cm⁻¹) (Fig. 6). The fruit from both 0 °C and 4 °C storage had higher $\mu_a 780$ values (0 °C, 0.048 ± 0.0006 cm⁻¹; 4 °C, 0.063 ± 0.001 cm⁻¹) than fruit after harvest (0.037 ± 0.0003 cm⁻¹).

When each treatment was examined daily during shelf life the kinetics were quite different at the two wavelengths, and among the treatments (Fig. 6). At $\mu_a 670$ fruit after harvest decreased in absorption as they ripened, as would be expected if the chlorophyll disappears during shelf life. Fruit after 0 °C began to decrease in $\mu_a 670$ for the first 3 d at 20 °C, and then this was reversed during the last 2 d of shelf life. Concomitantly, fruit from 4 °C storage increased in $\mu_a 670$ as shelf life was extended. The fruit without storage did not change in $\mu_a 780$ during 7 d at 20 °C. However, stored fruit, from either 0 °C or 4 °C, increased in absorption, with greater increases occurring after 4 °C storage.

Considering the seven categories of pulp defects (Fig. 7), it was found that $\mu_a 780$ discriminated healthy fruit (H) from those affected by defects. In addition, fruit with bleeding (BL) could be discriminated from those affected by browning (BR, BR+G, BR+BL+G). The $\mu_a 670$ was significantly lower in healthy and BL fruit, compared to values found in fruit with BR+BL, BR+G and BR+BL+G.

PCA based on the optical properties measured during shelf life after storage at 0 and 4 °C ($\mu_a 670$, $\mu_a 780$, $\mu'_s 670$ and $\mu'_s 780$) and the scores of each fruit regarding bleeding, browning and gel

Fig. 8. PCA of optical properties at 670 and 780 nm and scores of defects. Biplot of PC1 vs PC2 showing the loadings of variables and the scores of all fruits from shelf life of nectarines stored for 30 d at 4 °C or 0 °C. Symbols refer to the defect categories H, healthy; BR, internal browning; BL, internal bleeding; G, gel breakdown; BR+BL, BR+G, BR+BL+G, combinations of defects.

allowed the selection of two principal components (PC) which explained 70.60% of total variation (Fig. 8). In PC1 (47.53% of total variance) browning score (BR) and gel score (G) were positively related to $\mu_a 670$ and $\mu_a 780$, whereas in PC2 (23.07% of total vari-

Fig. 7. Absorption coefficients at 670 and 780 nm and number of fruit (N_{fruit}) of nectarines classified into 7 groups depending on flesh condition. The classes were: healthy (H), internal browning (BR), internal bleeding (BL), gel breakdown (G), BR+BL, BR+G, BR+BL+G. Columns with different letters are statistically different at the 0.05 level according to Tukey's test. Standard error is indicated.

Table 2

Correlation coefficients of absorption (μ_a) and scattering (μ'_s) coefficients at 670 and 780 nm measured during shelf-life at 20 °C with expressible juice (harvest (H), $n = 148$; after storage at 4 °C (ST4), $n = 145$; after storage at 0 °C (ST0), $n = 148$; pooled treatments, $n = 443$).

Treatments	μ_a 670	μ_a 780	μ'_s 670	μ'_s 780
H	−0.459***	−0.079 ^{ns}	−0.043 ^{ns}	−0.392***
ST0	−0.014 ^{ns}	−0.618***	+0.049 ^{ns}	+0.316***
ST4	−0.396***	−0.438***	+0.062 ^{ns}	+0.092 ^{ns}
Pooled	−0.364***	−0.559***	−0.049 ^{ns}	+0.078 ^{ns}

*** Significance of r : $p \leq 0.001$.

ance) μ'_s 670 and μ'_s 780 were selected. Both functions were able to distinguish fruit belonging to the various categories of pulp defects. PC1 distinguished healthy (H) fruit from those affected by pulp defects, with H category fruit having the lowest mean score (mean $\pm$ standard error, -1.48 ± 0.05); BL category differed from BR + G, BL + BR and BL + BR + G ones, having BL category a negative score (-0.61 ± 0.09) and the others positive scores, that is: BR + G, 1.21 ± 0.24 ; BL + BR, 1.27 ± 0.19 and BL + BR + G, 2.56 ± 0.27 . PC2 was able to distinguish BR + G category (1.15 ± 0.30) from H (-0.11 ± 0.10), BL (-0.27 ± 0.15) and BL + BR (-0.05 ± 0.23). In addition, all the categories having gel, i.e. G, BR + G and BL + BR + G, showed positive scores, in contrast with the other categories, all having negative mean scores.

From the study of the correlations between expressible juice and optical properties, a different scenario was found for each treatment (Table 2). At harvest there were significant negative correlations with μ_a 670 and μ'_s 780; after storage at 0 °C, a negative correlation was found with μ_a 780 and a positive one with μ'_s 780. On the other hand, after storage at 4 °C significant negative correlations were found for μ_a 670 and μ_a 780.

3.5. Canonical discriminant analysis

Canonical discriminant analysis based on seven selected attributes: μ_a 780, μ_a 670, weight loss, firmness, expressible juice, L^* of the non-blush side and C^* of the blush side of the fruit, indicated that the Canonical function 1 (Can1) accounted for 71.5% of the variation in the 7 variables of the three treatments during the last two days of shelf life, and the remaining 28.5% variation was accounted for by Canonical function 2 (Can2). In other words, two Canonical functions accounted for almost all the variation.

Can1 separated fruit without cold storage (20 °C fruit) from cool stored fruit and Can2 separated fruit cool stored at 4 °C from those cool stored at 0 °C (Fig. 9). Total-sample standardized canonical coefficients (an indication of relative importance of the selected attributes for discriminating) that Can1 separated fruit based predominantly on the contrast between μ_a 780 and μ_a 670, followed by weight loss, expressible juice and firmness. Fruit without cold storage had low μ_a 780, weight loss, and firmness, but high μ_a 670 and expressible juice compared with cool stored fruit. Can2 separated fruit based predominantly on μ_a 780, followed by firmness, weight loss and expressible juice. Fruit cool stored at 4 °C had high μ_a 780 and expressible juice, but low weight loss and firmness compared with fruit cool stored at 0 °C. The contribution of the last two variables, L^* and Chroma, were very small for both Can1 and Can2.

4. Discussion

Sorting nectarines at harvest into maturity levels using TRS measurement of μ_a 670 was shown to be an effective way of predicting their softening kinetics. Two models were developed, one for the decrease of μ_a (Tijskens et al., 2006) and one for fruit softening (firmness) (Tijskens et al., 2007), based on the assumption that

Fig. 9. Canonical discriminant analysis of all fruit from the last 2 d of shelf life of nectarines without storage (day 6 and 7), and nectarines stored for 30 d at 4 °C or 0 °C (day 4 and 5).

these two processes are highly coupled or synchronized, so that the biological shift factor for μ_a and the one for firmness can be mutually exchanged by a linear relation. The model thus included the variations in maturity at harvest of the individual fruit. The absorption coefficient at 670 nm measured at harvest was used in the model to describe the maturity at harvest and the future behavior in terms of firmness, e.g. to predict the rate of softening and thus the duration of shelf life (Rizzolo et al., 2009). The TRS measurement coupled with the biological shift factor theory was successfully applied in an export trial from Italy to The Netherlands, simulating on a small scale the fruit supply chain from the packing-house to the consumer (Eccher Zerbini et al., 2009).

In the current study μ_a 670 was used to group the nectarines into replicates that contained the whole variation of the maturity in each replicate. This allowed an investigation of the storage disorders to be focused on the interaction of storage temperature and fruit maturity. The results indicated that the more mature fruit were more susceptible to internal disorders than the less mature fruit. However, the spread of maturities in this study was much less broad than in previous ones. The distribution of μ_a measured at harvest showed a range much smaller ($0.15\text{--}0.03\text{ cm}^{-1}$) than that found in the early cv. Spring Bright ($0.35\text{--}0.03\text{ cm}^{-1}$) (Tijskens et al., 2006).

In the present study, storage disorders developed during ripening following storage at either 0 or 4 °C. However, there were some differences in the type of storage disorders and their intensity in fruit from the two storage regimes, with no gel development in fruit stored at 0 °C and very little internal browning. Fruit at 4 °C had both of these disorders. Bleeding in the flesh was also lower in fruit from 0 °C than from 4 °C. The development of internal disorders, such as internal browning or bleeding significantly influenced both the absorption coefficients, but mainly μ_a 780. In fact, in agreement with previous results on nectarines, μ_a 670 decreased as fruit ripening increased (Tijskens et al., 2006): in non stored fruit the decrease was till the end of shelf life, and in those stored at 0 °C till day 3, when there was an increase in bleeding and internal browning for this treatment. In contrast, in fruit stored at 4 °C, the appearance of internal browning already at day 1 of shelf life did not allow us to follow the decrease of μ_a 670 in shelf life due to the chlorophyll-a breakdown, but instead caused an increase in both μ_a 670 and μ_a 780. The fact that the increase in μ_a 780 in shelf life observed in nectarines could be due to the development of internal browning is in agreement with what was found in Granny Smith apples, where μ_a 750 increased and μ'_s 750 decreased with the development of internal browning (Vanoli et al., 2010). As for gel, it could be assumed that it would influence the absorption and scattering

coefficients at 780 nm, similarly to what found for watercore in Fuji apples (Vanoli et al., 2010), where healthy 'Fuji' pulp was characterized by lower μ_a 790 and higher μ'_s 790 than tissue affected by watercore. In Morsiani 90 nectarines, a clear relationship between μ'_s 780 and gel was not found, probably due to the very low number of fruit grouped (only 2) in the G category. In most cases gel was accompanied by either browning (BR + G), or by browning and bleeding (BR + BL + G). However, PCA results suggest that gel breakdown either alone (G) or associated with browning (BR + G) or with browning and bleeding (BL + BR + G) could be related to the reduced scattering coefficients at 670 and 780 nm.

Morsiani 90 is a freestone late-season variety with non-melting flesh. According to Brovelli et al. (1998) flesh woolliness as a result of chilling exposure in non-melting peaches is not as severe as is the case with melting fleshed peaches. In Morsiani 90 nectarines, the visual evaluation of symptoms of woolliness, defined as a coarse texture associated to a dry appearance, was difficult probably due to the very slight symptoms, which resulted in visible juice on the cut surface of the fruit, and with very slight pulp disruption and pastiness. However, the expressible juice trends of stored nectarines at both temperatures indicate a development of woolliness whatever the storage temperature.

It is known that fruit softening is accompanied with changes in the tissue density, weakening of cell–cell adhesion and changes in cell wall polymers, all of which may affect light scattering. Soft fruit have broader scattering profiles than firm fruit (Peng and Lu, 2004; Qin and Lu, 2008). Woolliness development causes changes to the cell wall polymers and decreases the amount of apoplastic liquid in the tissue (Lurie et al., 2000, 2003). These alterations would be expected to change the scattering profiles in a different way than normal softening.

Our results indicate that optical properties correlated to expressible juice differently depending on the treatment. At harvest, when no CI occurred and expressible juice increased with shelf life, there were negative relationships with μ_a 670, which decreases with ripening due to chlorophyll-a breakdown (Tijssens et al., 2006), and with μ'_s 780, which decreases with fruit softening similarly to what was found by Qin and Lu (2008). After storage at 0 °C, by plotting expressible juice against μ_a 780, it was found that higher values of μ_a 780 were found in fruit having expressible juice lower than 30% and bleeding and browning scores greater than 2 (data not shown). Nectarines with this type of CI were found at the end of shelf life, and more advanced in ripeness. These fruit had lower values of μ'_s 780, and a positive correlation between expressible juice and μ'_s 780. After storage at 4 °C, for μ_a 670 > 0.1 cm⁻¹ and μ_a 780 > 0.080 cm⁻¹ only browned fruit with expressible juice less than 30% could be found. So, when there was a less severe incidence of CI symptoms, as in the case of fruit stored at 0 °C, there was a correlation, even if weak, between woolliness as measured by expressible juice and μ'_s 780. On the other hand, when there was a severe incidence of CI symptoms, as in fruit stored at 4 °C, μ'_s did not show any correlation with woolliness development, due to the influence of the high absorption values related to internal browning which could have affected the estimation of scattering properties.

Canonical discriminant analysis was able to separate the fruit from all three treatments on the last 2 d of shelf life. These were the days when differences in flesh quality were the most apparent. Of the seven attributes found to be relevant, two of them relating to peel colour contributed only in a minor way to the analysis and could be ignored. The other five were μ_a at both wavelengths, expressible juice, firmness and weight loss. The separation of fruit from 0 °C or 4 °C was mainly due to differences in μ_a 780, weight loss and firmness. Absorption at 780 nm was 0.02 cm⁻¹ higher in fruit from 4 °C storage compared to 0 °C, weight loss was less and fruit were softer at 4 °C than at 0 °C.

This is the first study which shows the ability of an optical non-destructive technique, such as TRS, to determine nectarine storage disorders. Both wavelengths, 670 and 780 nm were able to differentiate between healthy and fruit with either woolliness, internal browning or internal bleeding. The major differences have been found for the absorption coefficients and were greater at 780 than at 670 nm. The light scattering at 670 nm did not differentiate between healthy and disordered tissue, whereas μ'_s 780 could differentiate between fruit with high expressible juice and those having low values of expressible juice. Future work will try to increase the sensitivity of the measurements in order to detect differences between healthy and disordered tissue at an earlier time in the ripening process.

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