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<u>Abstract</u>

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Introduction

Peaches are summer fruits, highly appreciated by consumers for flavor and juiciness, which can be used as fresh cut product. However, peaches fresh cut have a short shelf life that is the main limiting factor of their commercial diffusion. The most important problems are the browning of cut surface and rapid loss of firmness (Gorny et al. 1999). Browning is a direct consequence of polyphenol oxidase and peroxidase activity (Richard-Forget and Gauillard, 1997) while tissue softening is linked to ripening process. During fruit ripening, large changes occur in both pectins and matrix glycans, in fact pectins become increasingly de-esterified, leading to loss of integrity of cell walls, decrease of cell to-cell adhesion, increase of intercellular spaces and a change of tissue structure (Ortiz et al., 2011).

The qualitative decay depends on the respiratory metabolism, the aim of modified atmosphere packaging is to reduce the rate of respiration and water loss, and consequently to prolong the storage period. Low O_2 and elevated CO_2 atmospheres can extend the shelf life of many whole commodities (Kader, 1986) but may also be useful to minimally processed fruit. Studies on pear (Gorny *et al.* 2002), apple (Soliva-Fortuny *et al.*, 2004), mango (González-Aguilar *et al.*, 2000) and peach (Gorny *et al.*, 1999) fresh cut, showed the efficacy of MAP in maintaining high organoleptic characteristics. The

The effects of low O_2 and CO_2 -enriched atmospheres associated to different packaging (traditional and compostable) on shelf life of fresh-cut peach were investigated. The low permeability of the film has a positive influence on weight loss and firmness as the less permeable film allowed a greater water retention, which caused a lower weight loss of the samples. The MAP reduced respiratory metabolism with positive effect on color, total soluble solids, titratable acidity, firmness and PPO activity, however the efficacy was different in the two cultivars considered. The comparison between films showed that biodegradable films can be a good alternative to the use of PE film, but the high permeability is a great limit. The use of MAP for short periods was positive, acting on the respiratory metabolism in all parameters considered.

effects were to delay the decrease in titratable acidity values and to maintain the fruit flesh firmness, total soluble solids (TSS) and vitamin C content (Saltveit, 2003).

Traditionally for packaging purpose are used plastic film obtained from the petrochemical industry, but at present in food packaging field, compostable and biodegradable materials can be considered as an alternative to traditional plastics because they offer a partial solution to the problem of accumulation of solid waste. The objectives of this work were to investigate the effects of low O₂ and CO₂-enriched atmospheres associated to different packaging, traditional and compostable, on shelf life of fresh-cut nectarine slices stored (1°C) for 7 days.

Material and methods

Fruit material

In the study were used nectarine cvs 'Orion' and 'Sweet red', the fruits, of uniform size and defectfree, were harvested at firm-ripe stage (Orion: TSS 9.4 °brix; TA 138 meq/L; Sweet Red: TSS 9.2; TA 86.96) from a commercial orchard in Piemonte (Italy). Before treatment, the peaches were stored at 4°C for 24 h. The fruits were cored and cut into 8 slices using a stainless steel knife.

Processing methodologies

Minimal processed fruits were packed under



2 different atmospheres (passive and active MAP), with 2 different films (Film 1 and Film 2).

The films used for packaging were:

Film 1 - multilayer polyethylene + ethylene vinylalcohol, 33 μ m thickness, O₂ TR 60 ml m⁻² day ⁻¹ atm⁻¹, WVTR 1,2 g m⁻²d⁻¹

Film 2 - Polylactic acid (PLA), 40 μm thickness, O_2 TR 480 ml m^-2 day $^{-1}$ atm^-1 WVTR 15,3 $\,g\,m^{-2}d^{-1}$

Active modified atmosphere was applied by flushing a gas mixture with 5% of O_2 and 5% of CO_2 . 30 bags each cultivar (15 active MAP, 15 passive MAP) were obtained (UNIMEC packaging systems, Italy). Each bag was a sample unit and was composed by 8 slices from different fruits. The sealed bags were weighted and stored at 1°C for 7 days.

Analysis

The fruits were analyzed for: gas concentration, weight loss, firmness, color, quality measurements, Polyphenol oxidase (PPO) activity.

Gas monitoring

With the aim to measure kinetics of respiration processes, the concentration of oxygen and carbon dioxide inside the packages was monitored daily by using a CANAL 121 (Vizag, Gas Analysis, France). A sample of 0.5 mL was automatically withdrawn from the headspace atmosphere with a pin-needle connected to the injection system. The CO_2 level was measured with an infrared sensor. The O_2 level was measured with an electrochemical sensor. The instrument was calibrated towards air. The changes in gas composition was measured at 1, 4 and 7 days of storage.

Weight loss

Weight loss was determined by weighting the bags at the start of the trial (0 time) and during storage (4, 7 days of storage). Values are reported as percent of weight loss per initial fruit weight.

Firmness

Fruit firmness was tested using a TA-XT2i Texture Analyzer[®] (Stable Micro System) equipped with a 5 kg load cell. The firmness was performed at 0, 4 and 7 days of cold storage at 1°C (20 slices per treatment). Slices were sampled directly from the storage room, warmed to room temperature (20°C) for 3 h and then the measurements were made using a 3 mm probe (SMS P/3). The probe penetrated 3 mm into the slice at a crosshead speed of 3 mm s⁻¹ (Cocci *et al.*, 2006). The force max (N) value of penetration was calculated.

Color measurements

Color analysis was performed at 0, 2, 4 and 7 days of cold storage at 1°C (20 slices per treatment). L^* , a^* and b^* values were determined at two points along each side of the cut surface using a Minolta chromameter (CR400, Minolta, Tokyo, Japan) calibrated with a standard white plate (Y = 92.80, x = 0.3132, y = 0.3193). Numerical values of a^* and b^* parameters were employed to calculate the Hue angle (h° = tan ⁻¹ $(b^*/a^*)^2$). The reported values are the mean ± SD of 40 determinations.

Quality measurements

Total soluble solids (°Brix) and titratable acidity (meq/L) were performed at day 0 and after storage (7 days) in triplicate using juice extracted from 24 slices peach sample (each treatment) blended at high speed in a tissue homogenizer. Soluble solids content was determined by a digital refractometer (Atago refractometer model PR-32; Atago Italia, Milan, Italy). Titratable acidity was measured by titrating diluted juice (1:10) using 0.1 mol•l⁻¹ NaOH by an automatic titrator (Compact 44-00; Crison Instruments, Modena, Italy).

PPO activity

Enzyme extraction

A portion of 50 g of peach slices was mixed with a buffer solution (1:1) at pH = 6.5 containing 1 M NaCl (Sigma-Aldrich Co.) and 5% polyvinylpolypyrrolidone (Sigma-Aldrich Co.). The mixture was blended and homogenized using an Ultra Turrax T25 (IKAs WERKE).The homogenate was centrifuged at 12,500 rpm for 30 min at 4°C (Centrifuge AVANTITM J-25, Beckman Instruments Inc.). The supernatant was collected and filtered through Whatman 1 paper (Whatman Intl.), and the resulting solution constituted the enzymatic extract, which was used for enzyme activity determination.

PPO activity measurement

Polyphenoloxidase activity was determined according to the method of Soliva-Fortuny *et al.* (2002). Enzyme activity was assayed spectrophotometrically by adding 3 mL of 0.05 M catechol (Sigma-Aldrich) and 75 mL of extract to a 4.5-mL quartz cuvette of 1-cm path length. The changes in absorbance at 400 nm were recorded every 5 s up to 3 min from the time the enzyme extract was added using a Beckman Du 530 spectrophotometer. One unit of PPO activity was defined as a change in absorbance of 0.001 per minute and milliliter of enzymatic extract immediately after extract addition.



Figure 1. Changes in gas concentration in different packaging of Sweet Red (a) and Orion (b) nectarines during storage (°C). Means of three replicates

The initial reaction rate was estimated from the linear portion of the plotted curve. All determinations were performed in triplicate.

Statistical analysis

Data were analyzed by analysis of variance, using statistical procedures of the STATISTICA ver. 6.0 (Statsoft Inc., Tulsa, OK, USA), the sources of variance being MA treatments or storage. Tukey's HSP test (honest significant differences) was used to determine significant differences among treatments means. Mean values were considered significantly different at $p \le 0.05$.

Results and Discussion

Gas concentration

A decrease in O_2 concentration occurred in peaches packed with film 1 in association with an increase in CO_2 concentration. By contrast, the application of PLA film showed a higher value of O_2 and a lower value of CO_2 content. It may be seen that at the end of the storage the O_2 concentration was higher than at the beginning of the trial and the CO_2 content was slightly higher than the initial one (Figure 1). This pattern was quite the same for both the cultivars. The composition of the atmosphere must be related with the permeability of the film, PLA is characterized to have a high permeability to O_2 (Chonhenchob *et al.*, 2007) and a relative low one to CO_2 while the permeability of film 1 to both gases is lower.

The aim of MAP, active and passive, is to reduce the respiratory metabolism and, as consequence, delay the loss of fruit quality. In cv Orion was observed a lower concentration in O_2 in passive MAP sample, associated with an higher content in CO_2 , in both films, suggesting a more intense respiratory metabolism compared to the samples packed in active MAP. In cv Sweet Red instead the efficacy of active MAP was quite limited.

Weight loss

Fresh cut products are affected by high level of weight loss because internal tissue are exposed as consequence of cuticle and skin cut. One purpose of using MAP is to maintain a high relative humidity in the packaging, so dehydration typically is not a problem, however water permeability of the packaging film is critical in influencing the weight losses. It is known the water vapour permeability of the PLA film packaging is very high (Koide and Shi, 2007), higher than PE film package. In our work, the weight losses observed were very limited in all samples, between 0.1 and 1.8 % (Figure 2). In both cultivars, peaches packed with film 2 showed higher weight losses than peaches packed with film 1, according to different authors (Guilbert et al., 1997; Koide and Shi, 2007; Giacalone and Chiabrando, 2013). Active MAP was effective in reducing weight losses in almost all samples. Only in cv Sweet red, Film 1 associated with active MAP did not give

Cultiva	Treatment	L*				h*			
		day 0	day 2	day 4	day 7	day O	day 2	day 4	day 7
Sweet	Film1 + passive MAP	73.6 n.s.	66.23 b	68.18 b	71.53 b	96.08 n.s.	91.2 c	91.54 bc	93.08 b
Red	Film 1 + active MAP	73.6 n.s.	72.54 a	73.7 a	74.03 a	96.08 n.s.	95.67 a	95.51 a	95.56 a
	Film 2 + passive MAP	73.6 n.s.	72.31 a	72.28 a	71.53 b	96.08 n.s.	94.1 ab	92.68 ab	92.35 b
	Film 2 + active MAP	73.6 n.s.	71.68 a	68.0 b	70.19 b	96.08 n.s.	93.1 bc	88.56 c	92.56 b
Orion	Film1 + passive MAP	69.36 n.s.	72.04 b	71.61 b	70.79 c	98.03 n.s.	95.28 b	95.45 n.s.	93.62 c
	Film 1 + active MAP	69.36 n.s.	72.79 b	73.99 ab	72.1 bc	98.03 n.s.	96.14 b	96.53 n.s.	95.82 bc
	Film 2 + passive MAP	69.36 n.s.	75.83 a	74.14 a	76.29 a	98.03 n.s.	98.58 a	96.47 n.s.	98.31 a
	Film 2 + active MAP	69.36 n.s.	70.75 b	73.5 ab	73.55 b	90.03 n.s.	97.9 a	96.11 n.s.	96.62 ab

Table 1. Evolution of Lightness (L^*) and Hue angle (h^*) values of Sweet Red and Orion nectarines during storage (7 days at 1°C)

Means followed by different letter show significant difference (p < 0.05) during storage among treatments for each storage time. Means of 15 replicates



Figure 2. Weight loss in Sweet Red (a) and Orion nectarines (b) during the storage (7 days at 1°C). Means of three replicates. Means having * are significantly different ($P \le 0.05$).

effective results.

Flesh firmness

The rate of fruit softening during cold storage is proportional to the storage temperature, days of storage, genetic variability, ripeness and respiration rate (Fernandez-Trujillo and Artes, 1997; Toivonen and Brummell, 2008). The cause of softening is the increase of respiration rate and the high production of endogenous ethylene, which are a consequence of minimal processing. Ethylene production can promote ripening process and degradative changes in membranes and cell walls. Stress activates ACC synthase and ethylene production (Yu and Yang, 1980), ripening can be rapidly initiated by wounding in pre-climacteric fruit (Starrett and Laties, 1993), and the rate of softening of fresh-cut fruit pieces is often markedly more rapid than in intact whole fruit (O'Connor-Shaw et al., 1994).

However, in fresh cut fruit, can happen that the firmness increase, but it is only an apparent increasing due to water loss. In fresh-cut products, water loss is rapid, due to the absence of a cuticle. When the loss is especially important, the flesh firmness became quite elastic (Giacalone and Chiabrando, 2013). In cv Sweet Red it was possible to observe a loss of firmness in almost all treatments. In peaches packed with film 1 (passive MA) and film 2 (active and passive MA), flesh firmness at the end of the storage was significantly lower than at the beginning, moreover the firmness was quite the same after 4 and 7 days of storage (Figure 3). By contrast, the association film 1 and active MA resulted effective, in fact in these packaging, peaches showed a high flesh firmness during all storage.

There was a different behavior in cv Orion, in this case all treatments results effective and flesh firmness was kept high in both film and MA (Fig. 3).

Color

In cv Sweet Red, active MAP, associated with film 1 showed, at the end of the storage, values of Lightness and Hue angle significantly higher than the other treatments. It means that nectarines maintained the initial color throughout the entire storage. The same film associated with passive MAP was not

Cultivar Treatment		Total soluble solids (°brix)		Titrable acidity (meql ⁻ ¹)		PPO activity (unit of PPO g ⁻¹ of f.m.)		
		Day 0	Day 7	Day 0	Day 7	Day 1 Day	7	
Sweet Red	Film1 + passive MAP	9.20 n.s.	9.8 n.s.	86.96 n.s.	93.92 n.s.	0.012 n.s.	0.017 n.s.	
	Film 1 + active MAP	9.20 n.s.	8.5 n.s.	86.96 n.s.	89.16 n.s.	0.023 n.s.	0.053 n.s.	
	Film 2 + passive MAP	9.20 n.s.	9.4 n.s.	86.96 n.s.	86.35 n.s.	0.007 n.s.	0.028 n.s.	
	Film 2 + active MAP	9.20 n.s.	9.7 n.s.	86.96 n.s.	93.65 n.s.	0.02 n.s.	0.019 n.s.	
Orion	Film1 + passive MAP	9.45aA	9.3bA	138.05aA	109.44 b A	0.12 a B	0.051 b A	
	Film 1 + active MAP	9.45aA	8.8bA	138.05aA	101.30 b B	0.149 a A	0.031 b B	
	Film 2 + passive MAP	9.45aA	9.0bA	138.05aA	94.54 bB	0.112 a B	0.032 b B	
	Film 2 + active MAP	9.45aA	9.3bA	138.05aA	96.86 b B	0.109 a B	0.027 b B	

Table 2. Changes in total soluble solid content (°Brix), titratable acidity (meq l⁻¹) and PPO activity of fresh-cut nectarines packed in different MAP and stored 7 days (1°C)

Minor and capital letters show significant difference (p < 0.05) during storage for each treatment and among treatments for each storage time, respectively. Means of 3 replicates.



Figure 3. Changes in firmness of fresh-cut Sweet Red (a) and Orion (b) nectarines during storage (7 days at 1°C) in active/passive MAP. Means followed by the same letter are not significantly different at $P \le 0.05$ level. Means of 15 replicates ±SD

equally effective and fruits were dark after 4 days of storage. Film 2 associated with passive MAP gave good results until the fourth day of storage, while the association with active map wasn't enough effective (Table 1).

In cv Orion instead the best results were found for fruits packed with film 2 associated with passive MAP, while film 1 in association with passive MAP showed fruits with lower lightness and Hue angle. Active MAP wasn't effective and did not improve the color. Moreover, for a short period it seems that the use of MAP little affects the final results and that the overall trend was mainly dependent from the use of plastic film.

Total soluble solids, titratable acidity

After storage, total soluble solids (°Brix) and titratable acidity (meq/L) were analyzed revealing that the biggest difference was related to the length of storage. In cv Orion there was a decrease of the values of TSS and TA, and at the end of the trial both values

were significantly lower than at the start. While in cv Sweet Red there wasn't a significant loss of quality attributes (Table 2). The difference between the two cultivars could be attributed to the different stage of ripeness (cv Orion riper than cv Sweet Red).

In cv Sweet Red the association with active and passive MA was effective to reduce quality losses, the observed value along the storage period weren't statistically different, while in Orion peaches there was a physiological reduction in sugars and acidity content. The obtained results are in agreement with Akbudak and Eris (2004) that attributed to MAP an inhibition of fruit ripening in peaches less ripe.

PPO activity

A consequence of minimal processing of fruit is the loss of integrity of tissue, the compartmentalization of the cells is compromised and phenolic compounds, usually in vacuole, come into contact with PPO, this results in fruit browning because enzymatic browning reactions in fruits are catalysed by PPO. The intensity

of browning is influenced by the amount of active forms of the enzyme and by the phenolic content in the fruit tissue. In most fruits, the levels of phenolic substances are dependent on numerous factors, such as variety, maturity state, or environmental factors (Macheix, et al., 1990). In cv Orion PPO activity decreased during the storage in all treatments, the final value observed was between 20% and 40% of the initial one (Tab. 2) and this is in agreement with Murata et al., 1995. There was a significant difference among treatments: peaches in film 1 + passive MA showed, at the end of the storage, a higher enzymatic activity than the other treatments, for which were found quite similar values. Several authors (Gorny et al., 1999; Day, 1994) report the efficacy of active MAP in reducing PPO activity and the inactivation of enzymatic activity was associated with a reduction in browning of cut surface. In this case, we observed only a weak effect of active MA in relation with film 1, while for film 2 active MA had no effects. Different behaviour was observed in cv Sweet Red for which there were no difference among the treatments and during the storage (Table 2).

Conclusion

The effects of low O_2 and CO_2 -enriched atmospheres associated to different packaging effectively modified quality of peach slices stored (1°C) for 7 days. The low permeability of the film 1 has a positive influence on the parameter of weight loss and firmness as the less permeable film allowed a greater water retention, which caused a lower weight loss of the samples. The weight losses observed was very limited in all samples, between 0.1 and 1.8 %.

In cv Sweet red during storage, total soluble solids and titratable acidity have not significantly changed; the inhibition of ripening was the effect of the active and passive modified atmosphere which acted on the reduction of respiration rate of the slices. The color analysis showed that the best result of Hue angle and Lightness was recorded with Film 1 + active MA for Sweet Red while for Orion peach was better film 2 + passive MA . The values of PPO activity were very low in all tests and this is in agreement with many authors, which attributed to MAP a reduction of PPO activity. While in Sweet Red there were no significant differences between samples, in Orion was observed an effect of active MA in relation with film 1 (day 1).

In conclusion, the comparison between film 1 and film 2 shows that the films based on biodegradable substances can be a good alternative to the use of PE film, and that the main obstacle to the diffusion is the high permeability, which causes a greater weight loss of the product. The use of MAP for a short storage period was positive, acting on the respiratory metabolism in all parameters considered. Also a positive effect of the active MAP has been observed for the Orion cv rather than Sweet Red Cv probably due to the different stage of fruits ripeness.

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