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Evolution of respiration rate, internal CO_2 or O_2 and resistance to gas diffusion of anaerobic exposed and waxed 'Miho' satsuma fruits during market life

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Abstract: 'Miho' satsumas received an anaerobic treatment with N_2 or CO_2 for 24 hr at 20°C before being waxed with shellac and transferred to 20°C for 30 days, in order to measure respiration rate, internal gas evolution (CO_2 and O_2) and peel resistance to CO_2 diffusion. Ethanol in the juice, as well as taste and appearance were also determined. Respiration rate decreased by more than half following 30 days of storage, while internal CO_2 and O_2 rose and decreased, respectively. Conversely, resistance to CO_2 diffusion increased drastically with time in storage. Waxing significantly decreased respiration rate and doubled resistance to CO_2 diffusion, while anaerobic treatments did not influence physiological parameters. Off-flavour development due to time in storage or wax application strongly affected taste of fruits. Exposition to CO_2 negatively influenced fruit taste also, with respect to control and N_2 exposed fruits. In general, the higher the ethanol content in the juice, the higher the off-flavour score and the lower the acceptability by panellists. The external fruit appearance declined with time in storage. Waxing or anaerobic treated fruits maintained a better freshness than control fruits. From a commercial standpoint the best result was gained with non-waxed nitrogen-treated fruits, which stored satisfactorily for 10 days.

1. Introduction

Although satsuma "Wase" fruits ripen early in our climates when no other fresh citrus are available, they undergo a quick senescence, mainly as peel shrivelling and loss of taste and are prone to fungal spoilage if not promptly and adequately stored. Literature reports satsuma can be satisfactorily cold stored at 5°C and 90-95% relative humidity (RH) for four to six weeks (Beever, 1990), while other cultivars, like clementines, are waiting to be harvested and transported to markets.

Means to substitute, limit or improve cold storage effectiveness on citrus and other fruit species include controlled atmosphere, plastic packaging and application of coatings. The beneficial effects of traditional controlled atmosphere can be observed, from a commercial point of view, only on apple and pear fruit (Little *et al.*, 1982; Ke *et al.*, 1990), while short-term exposition to low oxygen atmosphere (with N_2 and CO_2 as complement gases) resulted in a decrease in acid content of orange and grapefruit juice (Bruemmer and Roe, 1969) or in decay reduction, both in cold storage and shelf-life (SL) conditions on several citrus species (Pesis and Avissar, 1988). Recently, Piga *et al.* (1997) found a positive effect in delaying loss of firmness and freshness of "Page" tangelos treated for 24 hr with a 99% N_2 + 1% O_2 atmosphere.

Film packaging or coating application can represent effective tools in maintaining quality of citrus fruit. In particular, appropriate plastic films drastically reduce fruit weight loss without modifying internal gas composition, while waxes or edible coatings enhance fruit appeal, can reduce spoilage risk (Grierson and Wardoski, 1978) and diminish weight loss as well, even if to a lesser extent than plastics. On the other hand, plastic packaging can result in an excessive decay spread (Miller and Risse, 1988) and waxing in off-flavour develop-

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ment, due to the increased skin resistance to gas diffusion, thus leading to an anaerobic metabolism with elevated acetaldehyde and ethanol build-up in the juice (Banks, 1984). Nevertheless, citrus fruit deterioration is slower than other fruit (such as pears, peaches or avocados) and low temperature benefits are not as important as for climacteric fruits. Several reports indicate the lack of significant chemical changes in citrus fruit stored at different temperatures (El-Zeftawi, 1976; Arras and Schirra, 1988), or the good quality maintenance in shelf-life of film wrapped 'Miho' satsumas (D'Aquino *et al.*, 1996) or 'Minneola' tangelos (D'Aquino, personal communication). Thus, the practice of citrus refrigeration can be sometimes replaced, chiefly for the fresh fruit market, with the above-cited alternative postharvest technologies, avoiding the need for cold storage facilities and related refrigeration energy.

In the present study we examined the effect of a short exposition to anaerobic conditions prior to waxing of satsuma fruits on endogenous CO_2 and O_2 concentrations, peel resistance to CO_2 diffusion and ethanol accumulation in the fruit juice, as well as on flavour and overall appearance of the fruit in order to determine whether these conditions may be tolerated and allow adequate storability of the fruit at market conditions.

2. Materials and Methods

Materials and treatments

Fruits of 'Miho' (*Citrus unshiu* Marc.) satsuma were harvested in mid-October and delivered within a few hours with a non-conditioned van to our laboratory, where they remained overnight at 20°C. The next morning, fruit with any external defect, that was overripe or out of size were culled and three lots of 900 fruits were placed inside as many as 250-l plastic cabinets, which were then hermetically sealed. Two holes in the topside of each cabinet served as entering and exiting ports for gas treatments. Water-saturated nitrogen or carbon dioxide at a flow rate of 4.5 l/min were introduced through Tygon tubes within 5 cm of the cabinet bottom and stopped when oxygen level reached 1%. Holes were then immediately sealed with silicone sealant. Treatments were performed for 1 hr and then the cabinets remained closed for 23 hr (at 20°C), while the control lot was flushed at the same flow rate with water-saturated air for 24 hr. After opening the cabinets, half of the fruit from each gas treatment received a wax application with shellac [supplied by Elf Atochem Agri Italia, Belpasso (CT), Italy] and then were air-dried in a heated air drier. Finally, fruits were placed in nine boxes per treatment, 50 fruits per box and held 30 days at 20°C and 70% of RH. All the following evaluations were performed at 10, 20 and 30 days of the holding period by taking at random fruits from three boxes.

Gas measurements

The CO_2 and O_2 concentrations inside cabinets were measured by withdrawing from the exit port 20 ml samples which were injected into an infrared CO_2 analyser connected in series with a paramagnetic O_2 analyser (Servomex 1450B3). Respiration rate (Rr), expressed as CO_2 production, was measured by taking 2 ml gas samples from the headspace of 1-l jars fitted with rubber serum stoppers, in which fruits were individually closed for 1 hr at 20°C. The previous methodology of detection was used. Samples of 2 ml of the internal fruit atmosphere were diluted to 20 ml with air and measured for CO_2 (Cc) and O_2 (Co) percentages with the respective analyser. Precision of the method was assessed by using the same dilution technique with gas samples of known concentrations. Resistance to CO_2 diffusion (r_{CO_2}) was calculated with the ratio reported by Trout *et al.* (1942): $[(CO_2)_{Internal} - (CO_2)_{External}]/CO_2$ production rate] and expressed as $[\%\cdot(ml\cdot kg^{-1}\cdot h^{-1})^{-1}]$. We measured only r_{CO_2} as it is easier to determine CO_2 rather than O_2 consumption and they are usually similar, although not identical. The same fruits (six for each treatment) were used for (Rr) production rate and r_{CO_2} determinations.

Flavour scoring

An informal panel test of five trained technicians subjectively ranked five fruits per treatment for sweetness (Sw), sourness (Sr), off-flavour (Of) and acceptability (Ap) on a scale of 1 to 10, with 1=low intensity and 10=extremely high intensity.

Ethanol

Ethanol in the headspace of fruit juice was determined using a Varian 3300 gas chromatograph fitted with a FID detector and equipped with a 6.6 Carbowax 20M on 80/120 Carbograph 1AW glass column as described by Piga *et al.* (1997).

Overall appearance

Panellists evaluated the external appearance (Oa) with a subjective scale of 1 to 5 where 5=excellent (fruit at harvest), 4=good, 3=fair (limit of marketability), 2=moderate shrinkage, 1=severe shrinkage.

Statistical analysis

Data were analysed as a 4x3x2 completely randomised factorial design, where the main factors were holding time, treatments and waxing, respectively. Means were separated by the Duncan's Multiple Range Test at 0.05, 0.01 and 0.001 level of significance, as appropriate. The M-STAT software (Michigan State University, 1991) was used.

All fruits showing decay were discarded in order to not affect the physiology of healthy fruits.

3. Results and Discussion

Respiration rate, internal CO_2 and O_2 and resistance to gas diffusion

Respiration rate strongly declined during the holding time at 20°C. In fact, Rr more than halved at 30 days after

Table 1 - Effects of holding period, treatments and waxing on CO_2 production rate, internal CO_2 and O_2 and resistance to CO_2 diffusion of 'Miho' satsumas

Source of variation		CO_2 production rate ($ml \cdot kg^{-1} \cdot h^{-1}$)	Internal O_2 (%)	Internal CO_2 (%)	r_{CO_2} [$\% \cdot (ml \cdot kg^{-1} \cdot h^{-1})^{-1}$]
Holding period (P)					
harvest		28.01 a[z]	18.80 a	1.63 b	0.057 d
10 days		17.38 b	17.50 b	2.30 b	0.131 c
20 days		13.21 c	15.20 c	3.27 a	0.249 b
30 days		11.69 d	14.10 d	3.63 a	0.333 a
Significance		***	***	***	***
Treatment (T)					
Control		17.65 a	16.40 a	2.68 a	0.190 a
N_2		17.21 a	16.31 a	2.75 a	0.197 a
CO_2		17.85 a	16.49 a	2.69 a	0.189 a
Significance		NS	NS	NS	NS
Waxing (W)					
Nonwaxed		17.99 a	16.98 a	1.99 b	0.129 b
Waxed		17.15 b	15.82 b	3.43 a	0.256 a
Significance		**	***	***	***
			Interactions		
	df				
P x T	6	NS	NS	NS	NS
P x W	3	**	***	***	***
T x W	2	**	NS	*	NS
P x W x T	6	*	NS	**	*

[z] Values in a column within each group variable followed by different letters are significantly different.
[NS], *, **, *** Non significant or significantly different at $P \leq 0.05$, 0.01 and 0.001, respectively.

harvesting (Table 1). Conversely, Cc significantly rose during the holding period, being mirrored by a drastic diminution in the Co level and in a six-fold higher r_{CO_2} at the end of the 30-day interval. The application of wax also lowered the Rr and affected the Cc increment more than the Co diminution, thus enhancing r_{CO_2}. The short anaerobic exposition with CO_2 or N_2 seemed to have not affected the above-cited parameters, although a slightly higher Rr was recorded for CO_2 treated fruits. Among others, a significant interaction was found for holding period *vs.* waxing for all these parameters (Table 1). An increase in Cc and diminution in Co, accompanied by a decline in Rr, clearly indicates a rise in resistance to diffusion of gas. Our data on r_{CO_2} confirm this suggestion. Ben-Yehoshua (1969) stated that increased resistance to gas diffusion in oranges was due to drying of the peel due to transpiration. Several bodies of evidence seem to confirm this in our study. Figure 1 shows a gradual increase of Cc and r_{CO_2} or decrease of Co both for waxed and non-waxed fruit according to the time in storage, along with progressive shrivelling of the peel. Moreover, holding fruit at different RH reduced both dryness of the peel and Cc for fruits stored at higher RH (Ben-Yehoshua, 1969). Wrapping, which is beneficial for reducing transpiration and consequently peel shrinkage (citrus moisture loss is chiefly from the peel) result-

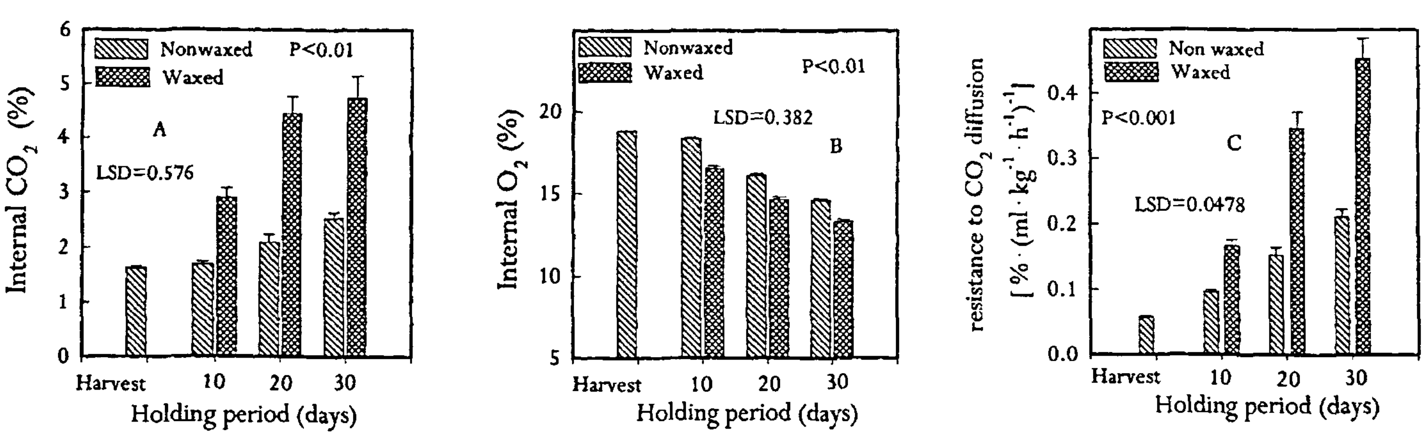

Fig. 1 - Effect of waxing on changes in internal CO_2 (A), O_2 (B) and peel resistance to CO_2 diffusion (C) during storage of 'Miho' satsuma fruits at 20°C and 70% RH. Means are the average of six measurements. Vertical bars indicate SE.

ed in a lower Cc and both higher Co and Rr than non-wrapping in satsuma fruits (D'Aquino *et al.*, 1997). Application of wax confirmed the results shown in previous reports (Banks, 1984; Ben-Yehoshua, 1985). In fact, although coating with wax reduces peel drying, there is a rise in Cc depending on the number of pores plugged with the coating and the degree of adhesion both near the pore area and the cuticle, the two routes in which gas diffusion is allowed (Banks *et al.*, 1993). In fact, Ben-Yehoshua (1985) reported an increase of resistance after waxing "Valencia" oranges of 140 and 250% for CO_2 and O_2, respectively, if compared to non-waxed fruit, while resistance to transpiration was increased by only 25%.

Flavour scoring, ethanol accumulation and overall appearance

Tasting of fruits revealed a decrease in Sw and Sr as the holding period increased (Table 2). In particular, there was a drastic diminution of the above-cited parameters at the end of the first ten-day storage period, while in the other two periods there was a more gradual decrease. Loss of Sw and Sr is undoubtedly to be ascribed to sugar and acid demolition by respiratory metabolism. Neither treatments nor wax affected Sw and Sr, while CO_2 and wax application negatively influenced the overall acceptability, as panellists indicated a stronger off-flavour in these fruits, with respect to the non-waxed and N_2-treated ones (Table 2). Negative effects of waxing, such as off-flavour development have been reported on citrus and other fruit species (Ben-Yehoshua, 1967; Cohen *et* al., 1990). Detrimental flavour changes in waxed fruits can be attributed to the previously cited increased resistance to gas, thus resulting in anaerobic respiration by CO_2 build-up inside fruits. However, the off-flavour score was also time-dependent. The progressive drying of the peel during the holding period and the concomitant (Cc) rise (see above) can explain this off-flavour trend. Concerning ethanol amounts in the juice, CO_2-exposed fruits showed the highest ethanol content among the treatments (Table 2). Similar results were obtained with 'Valencia' oranges (Pesis and Avissar, 1988). Although CO_2 and N_2 were applied under the same conditions, ethanol was significantly higher in the former fruits with respect to the latter, probably because CO_2 concentration can be utilised in the metabolism by PEP carboxylase (Pesis and Ben-Arie, 1986) while N_2 exposition can only give anaerobic conditions. We found a particular trend in endogenous ethanol during the holding period. In fact, we registered a more than four-fold increase after 10 days in SL, then ethanol content dropped at the end of 20 days and later on it peaked by the last holding period. The higher concentration at 10 days, if compared to 20 days, could be ascribed to an accumulation, following anaerobic exposure, of ethanol, which subsequently would be in part lost by evaporation during the second holding period. The ethanol peak at 30 days could be explained both with a reduced peel permeability to this compound and endogenous CO_2 accumulation, which

Table 2 - Effects of holding period, treatments and waxing on taste parameters, overall visual appearance and ethanol content of the juice of 'Miho' satsumas

Source of variation	df	Sweetness	Sourness	Off-flavour	Acceptability	Overall appearance	Ethanol (µl/l)
Holding period (P)							
harvest		8.00 a	7.00 a	1.00 c	8.00 a	5.00 a	55.00 d
10 days		6.50 b	5.61 b	2.50 b	5.89 b	4.03 b	224.05 b
20 days		6.00 c	5.00 c	2.61 b	5.19 c	2.94 c	169.56 c
30 days		5.53 d	4.75 d	3.00 c	4.69 d	2.32 d	251.00 a
Significance		***	***	***	***	***	***
Treatment (T)							
Control		6.48 a	5.60 a	1.67 b	6.29 a	3.36 b	127.62 c
N_2		6.56 a	5.65 a	1.77 b	6.13 a	3.71 a	173.79 b
CO_2		6.48 a	5.52 a	2.65 a	5.42 b	3.72 a	223.29 a
Significance		NS	NS	***	***	**	***
Waxing (W)							
Nonwaxed		6.53 a	5.65 a	1.58 b	6.15 a	3.47 b	133.28 b
Waxed		6.49 a	5.53 b	2.47 a	5.74 b	3.72 a	216.53 a
Significance		NS	*	***	***	***	***
Interactions							
P x T	6	NS	NS	***	***	***	***
P x W	3	NS	*	***	***	***	***
T x W	2	NS	NS	***	***	**	NS
P x W x T	6	NS	NS	***	***	**	***

[z] Values in a column within each group variable followed by different letters are significantly different.

[NS], *, **, *** Non significant or significantly different at P≤ 0.05, 0.01 and 0.001, respectively.

may have overcome the natural loss. It is well known that accumulation of ethanol in the flesh may lead to alcoholic off-flavour development in several fruits (Ke and Kader, 1990; Ke *et al.*, 1990). In our study, Ap (Table 2) was strongly influenced by off-flavour that was surely to be attributed to the ethanol accumulation since panellists detected an alcoholic taste. Thus, the lower the Ap, the higher the off-flavour score and ethanol amount in the juice, if the 20-day storage is excluded. Thus, according to the data regarding off-flavour and in part to ethanol content in the juice, Ap declined during the holding time, was lower in CO_2-exposed fruits if compared to N_2 and control fruit, and higher in non-waxed satsumas with respect to waxed ones (Table 2). After 20 days in storage only non-waxed control and N_2 fruits were judged as edible (data not shown). However, Ap was also negatively influenced by the decrease in sweetness and sourness scores, even if to a lesser extent than off-flavour. As expected, the Oa declined significantly with time in storage (Table 2). Shrinking of the peel, reduced brightness and loss of the stem end were recorded by panellists. Both anaerobic-treated and waxed fruit were rated better than control fruits. The beneficial effect of waxing is due to the enhanced peel gloss, while anaerobic treatments could have delayed the senescence of the fruit. Waxing, however, influenced markedly Oa until 20 days, when only waxed fruits were scored as marketable (Fig. 2).

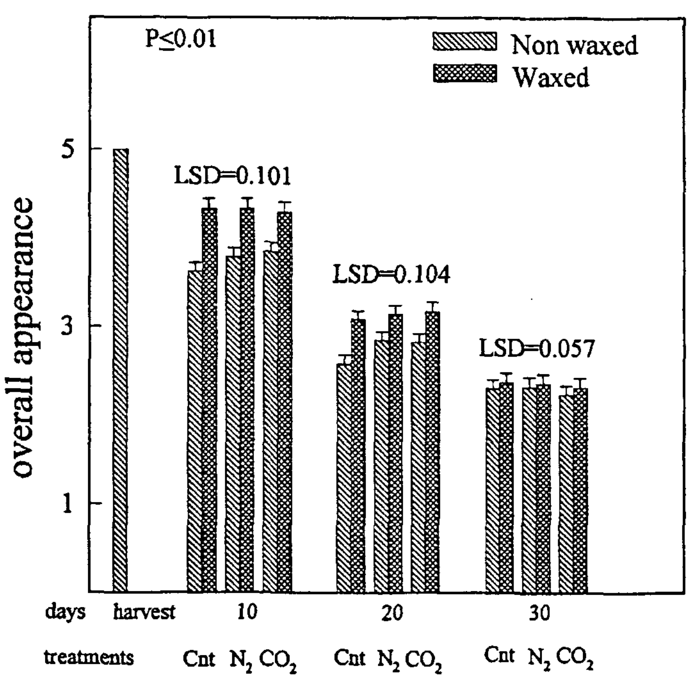

Fig. 2 - Overall appearance decline during storage of 'Miho' satsuma fruits at 20°C and 70% RH as affected by anaerobic treatments or was application. Means are the average of 100 measurements. Verticals bars indicate SE.

4. Conclusions

Results from our study show that both short anaerobic esposure and wax application prior to storage under simulated market conditions have negligible effects in maintaining quality of 'Miho' satsumas. The best combination matching external appearance and fruit taste, which was non-waxed nitrogen treated, allowed fruit quality to be maintained for 10 days only. Further attempts, such as storing at high relative humidity, N_2-exposed fruits or wrapping in plastic films, could be effective tools in extending the market life and maintaining quality of citrus fruits, provided there are appropriate fungicide applications, thus saving the cost of refrigeration.

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