

Carnauba Wax and Modified Atmosphere in Refrigerated Preservation of ‘Tahiti’ Acid Limes

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Peel yellowing and loss of fruit mass reduce the useful life of acid limes and limit their commercialization. The goal of the present study was to prolong the post-harvest preservation of acid limes using wax and packaging. Fruits were harvested, selected, disinfected, treated with fungicide, coated with Carnauba wax and placed in cardboard boxes wrapped with different plastic packaging. A fruit lot treated only with wax coating was used as a control. The fruits were stored at 10°C for 24 days followed by 6 days at 20°C. The gas atmosphere inside the packages was analysed during storage. The loss of mass, color index, titratable acidity, and total chlorophyll, ascorbic acid, soluble solids, acetaldehyde and ethanol concentrations of the fruits were measured. The combined use of wax coating and plastic Cryovac® D-955 (Cryovac Brasil Ltda, São Paulo, Brazil) and Vegetal Pack® (Eletropolimeros do Brasil Ltda, São Paulo, Brazil) films resulted in better post-harvest preservation of acid limes compared with only wax coating because the combined treatment maintained the green peel color following 30 days of storage in addition to resulting in lower loss of mass and maintaining chemical characteristics adequate for commercialization. The LDPE (low density polyethylene) and Xtend® packaging (Stepac Brasil Ltda, São Paulo, Brazil), although they prevented peel degreening, did not result in an adequate atmosphere to maintain the quality of limes due to the accumulation of high concentrations of acetaldehyde and ethanol, especially for the LDPE packaging. LDPE drastically changed the atmosphere surrounding the fruit, resulting in loss of the characteristic pulp color, in addition to conferring an alcohol odor. Copyright © 2015 John Wiley & Sons, Ltd.

Received 20 May 2014; Revised 7 November 2014; Accepted 1 March 2015

KEY WORDS: Citrus latifolia; degreening; loss of mass; post-harvest

INTRODUCTION

Although Brazil is one of the largest producers and exporters of ‘Tahiti’ acid limes worldwide, it exports only 5% of the total produced. One of the factors preventing the increase of acid lime export is the low fruit quality resulting from the loss of the green peel color, which is an extremely desirable characteristic that should be preserved throughout the entire post-harvest useful life. The total or partial appearance of yellow color decreases the acid lime acceptance by the consumer market and therefore limits the entrance of Brazilian limes into new international markets.¹

In commercial plantations, this fruit is harvested with green color after reaching full development, and it is commercialized while the peel remains green. However, the processes of chlorophyll degradation and carotenoid synthesis continue to occur during fruit commercialization under both ambient

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and refrigerated conditions, culminating with fruit degreening, which compromises lime commercialization due to the loss of commercial value.²

Refrigerated storage is an important tool for prolonging the useful life of acid limes. Refrigeration is commonly used for fruits destined for export; these fruits require at least 30 days of post-harvest useful life to enable the use of slower transportation means, which have lower costs, increasing competitiveness at the international market.³ 'Tahiti' limes can be maintained between 10 and 12°C, with relative humidity between 85% and 95%, from 4 to 8 weeks,⁴ according to the literature. However, even under these conditions and/or using adequate post-harvest processing procedures, the producers and exporters of these fruits have problems with the loss of green color and turgor, attributes that are essential for the consumer market.⁵

Some auxiliary refrigeration treatments, such as the use of packaging^{6,7} and the application of wax^{8,9} and growth regulators,^{2,10} have been tested or applied to 'Tahiti' limes, with the goal of maintaining the green peel color and prolonging the commercialization period of limes.

Modified atmosphere packaging is effective in extending the useful life of fruits and vegetables. However, such packaging has not been used as a commercialization tool for Tahiti acid limes. Regardless of its positive effects, if it is applied incorrectly, modified atmosphere packaging can result in negative effects during storage, such as the occurrence of physiological disorders, irregular fruit ripening, anaerobic respiration and rotting.¹¹ The correct use of modified atmosphere packaging for produce requires the optimization of physical, chemical, biochemical and environmental parameters and is therefore complex, requiring experimental studies¹².

Carnauba wax-based coatings and polyethylene wrappings are commercially used as post-harvest treatments in citrus, with the goal of decreasing loss of fruit mass and consequently fruit softening and wilting. In addition, wax coating confers a shiny appearance to acid limes, making them more attractive to consumers.¹³ However, the use of this post-harvest procedure at packing houses has not been adequate to maintain the quality of fruits destined for export, especially in terms of prevention of peel degreening and fruit mass loss.

The goal of the present study was to evaluate the combined effect of modified atmosphere packaging and wax coating for the maintenance of the green peel color of 'Tahiti' acid limes, preserved under refrigeration, with the goal of extending their storage period.

Materials and Methods

'Tahiti' acid limes, originating from commercial orchards from the Itajobi municipality (49°03'16" W and 21°19'05" S, 453 m), São Paulo, were harvested at the physiological maturation stage established by export-quality standards.¹⁴

Freshly harvested fruits were subjected to post-harvest procedures required for fruit export at the packing house of the Itacitrus company, consisting of disinfection with 1 ppm sodium hypochlorite solution; selection based on size, color and absence of mechanical damage; application of 200 ml/100 l Magnate 500 EC® (ADAMA Brasil, Paraná, Brazil) fungicide (50% imazalil); spraying with 1 kg/t AruáBR carnauba wax (18% soluble solids); and packaging in export-type cardboard boxes with 4.5 kg load capacity.

Immediately following processing, the limes packaged into cardboard boxes were carefully transported in a refrigerated vehicle to the Produce Post-Harvest Laboratory of the Department of Plant Production of the Luiz de Queiroz College of Agriculture (*Escola Superior de Agricultura Luiz de Queiroz-ESALQ/USP*), in Piracicaba, São Paulo (SP), where they were again selected based on the export quality standards, as previously described. The boxes, containing 4.5 kg fruits each, were then divided into six lots. Different types of plastic packaging were tested in five of the lots (Table 1). The remaining lot was kept in the export-type cardboard box with no lid or plastic packaging (Control).

For packaging, low density polyethylene (LDPE), Vegetal Pack®, and Xtend® plastic bags were placed inside the boxes prior to receiving the fruits (4.5 kg per box). The open sides of the bags were twisted and tied with elastic bands to minimize gas exchange with the external environment. Cryovac® D-955 plastic film was cut according to the size of the cardboard boxes, and after the fruits were placed in the boxes, the boxes were wrapped with the film, and the film was sealed and heat-shrunk using a hot air blower.

Table 1. Characteristics of the studied plastic packaging.

Plastic packaging	Thickness (μm)	Characteristics	Gas transmission rate*, 23°C, dry, at 1 atm	
			ml(STP)/m ² /day	
			O ₂	CO ₂
Cryovac [®] D-955	15	Polyolephin heat shrink film (40 × 60 cm)	9.760	35.331
LDPE ^a	30	Plastic bag (40 × 60 cm)	7.489	27.988
LDPE ^a	65	Plastic bag (40 × 60 cm)	4.650	16.139
Xtend ^{®b}	18	Laser microperforated plastic bag with lateral fold (47 × 60 × 12 cm)	48.21**	127**
Vegetal Pack [®]	18	LDPE bag with lateral fold impregnated with zeolite (47 × 60 × 12 cm)	9.185	41.108

^aLDPE: low density polyethylene.

^bCode: 815-LM8 – patent no. 6190710 – StePac – DS Smith.

*Oxygen transmission rate determined according to the procedure described in the norm ASTM D 3985–5,¹⁵ and carbon dioxide transmission rate determined using the increasing concentration method.¹²

**Measurement performed on a surface without micropores.

The fruits were stored at 10±1°C and 75±5% RH for 24 days and then under ambient conditions (20±1°C; 75±5% RH), without the plastic packaging, for an additional 6 days. At the end of the storage period, the following parameters were measured:

- Loss of mass*: determined as the difference, as a percentage, between the initial mass of each replicate (4.5 kg box) and the mass following treatments and storage.
- Ascorbic acid concentration*: determined by titulometry¹⁶ and expressed as mg/g.
- Soluble solids (SS) concentration*: determined by direct reading using a digital refractometer Atago Pallete–101 and expressed as °Brix.
- Titrateable acidity*: determined by titration using a pH meter, with 1 N sodium hydroxide, until pH 8.1 was reached¹⁶. The results were expressed as the percentage of citric acid in the juice.
- Total chlorophyll*: determined according to a modified method¹⁷, consisting of removing 1 cm² of peel from each fruit. Composite samples of peel were collected from 10 fruits and kept in 80% acetone solution for 72 h. The results were expressed as mg/g.
- Ethanol and acetaldehyde concentrations*: determined according to a modified method¹⁸ as follows: ethanol (50 to 4050 μg) and acetaldehyde (5 to 40 μg) standard solutions were prepared. 1 ml aliquots of the ethanol and acetaldehyde standards and of acid lime juice were placed in separate 40 ml hermetic flasks and incubated in a water bath at 50°C for 30 min. During incubation, 1 ml of air from each flask was collected using a gas-proof syringe and injected into a gas chromatographer equipped with a flame ionization detector and a Porapak N 1.8 m column. The following chromatography settings were used: 140°C for 8 min, gradient of 20°C/min up to 180°C, and isotherm of 2 min for column cleaning; injector at 150°C; detector at 180°C; 190 kPa pressure (constant); and 70 ml/min N₂ flow rate. The acetaldehyde and ethanol concentrations were calculated by comparing the chromatography peak areas with the peak areas obtained for the standard curve. The results were expressed as g/100 g.

The atmosphere inside the packages (O₂, CO₂ and ethylene) was monitored daily during the first 4 days and then every 4 days until day 24 (end of refrigerated storage). The CO₂ and O₂ concentrations inside the packages were quantified by collecting 2 ml aliquots from the gas atmosphere inside the package, using a gas analyzer PBI Dansensor Check Mate (Dansensor, Ringsted, Denmark), and expressed as percentages of O₂ and CO₂. The ethylene concentration was determined in 1 ml aliquots from the package atmosphere, injected into a gas chromatographer Thermo Fisher Trace GC 2000 (Thermo Fisher Scientific, Texas, USA) equipped with a flame ionization detector and a Porapak N column. Hydrogen was used as the carrier gas, with a flow of 30 ml/min. The analytical temperatures were maintained at 80°C for the column, 100°C for the injector, 250°C for the detector and 350°C for the methanator. The results were expressed as ppm.

The peel color index of limes was measured every 8 days during the 30 days of storage and 6 days after the transfer to ambient conditions, using a colorimeter Minolta CR-300 (KONICA MINOLTA, Tokyo, Japan). Two readings per fruit were performed at opposite sides of the fruit's equatorial section. The results were expressed as a color index (CI), which was calculated using the following equation: $CI = (1000 \times a)/(L \times b)$. More negative CI values indicate greener peel colors, and more yellow peel colors have more positive CI values. Zero corresponds to color tones halfway between green and yellow.¹⁹

The experimental design was completely randomized, with a factorial scheme (treatments \times storage time) with four replicates per treatment. Each replicate was composed of ten fruits taken from the 4.5 kg boxes. The results were subjected to a variance analysis, followed by a Tukey test, at $p < 0.05$.

RESULTS AND DISCUSSION

The modified atmosphere inside the packages reached equilibrium at the third day of refrigerated storage with the Cryovac® D-955, Xtend® and Vegetal Pack® packaging. However, for the LDPE packaging, equilibrium practically did not develop using either of the two tested thicknesses (Figure 1). The fast achievement of an equilibrated atmosphere inside the packaging is desirable because it maximizes the product's durability without compromising its quality³.

The LDPE bags of the two tested thicknesses drastically changed the atmosphere inside the packages, resulting in modified atmospheres with O₂ levels under 1% and CO₂ levels higher than 12% at the end of the storage period. These results differed from the modified atmospheres obtained using Xtend® (O₂ = 15.45%; CO₂ = 8.20%), Cryovac® D-955 (O₂ = 11.87%; CO₂ = 5.90%) and Vegetal Pack® (O₂ = 6.88%; CO₂ = 5.83%). The ideal O₂ concentrations for the preservation of 'Tahiti' acid lime have been reported to be between 5% and 10%, and the ideal levels of CO₂ range between 0% and 5%²⁰. Cryovac® D-955 and Vegetal Pack® were therefore observed to promote atmospheric gas levels close to those desirable to maintain fruit quality.

Ethylene concentrations inside the packages increased up to day 2, followed by a decrease between days 4 and 8 and an increase up to day 24 of the refrigerated storage, except with Xtend® and Vegetal Pack® (Figure 2). The Xtend® bags resulted in low ethylene concentrations (0.1 ppm) inside the package during the 24 days of refrigerated storage, differing from the atmosphere inside the Cryovac® D-955 and Vegetal Pack® packages, which presented intermediate levels (0.19 \pm 0.06 ppm and 0.27 \pm 0.05 ppm, respectively), and from the LDPE packaging, which presented high levels of ethylene from day 8. These differences may be attributed to differences in permeability between different packages, which are important information because ethylene levels starting at 0.1 ppm can induce yellowing of the peels of acid limes, which is a determinant factor for commercialization.²¹ This result indicates that LDPE packages are not adequate for the preservation of 'Tahiti' limes because the packages can accelerate fruit degreening.

The intensity of the green peel color decreased with all package types tested, except for LDPE, which maintained the green color (Table 2; Figure 3). However, this color loss occurred faster in the limes with only wax coating (Control). This pattern indicates that the combined use of wax coating and plastic packaging was more efficient in preventing peel degreening due to the atmospheric modification resulting from the treatment combinations.²² Additionally, 6 days following the removal of the plastic bags and under ambient conditions, the acid limes previously packed in LDPE bags (30 μ m) exhibited significantly greener peels than the ones packed in Cryovac® D-955, and the latter were similar to the limes in the Control, Xtend®, Vegetal Pack® and LDPE (65 μ m). However, although the results were statistically, significantly different, the fruits from all the treatments presented green color, and the color differences were subtle and not easily detectable by the naked eye.

Although the type of package affected the loss of intensity of the green peel color, no differences were observed in total chlorophyll content (Table 2). This result is in accordance with the measured color index, which indicated that the fruits, independently of the treatment, presented predominantly green peel colors (Figure 3).

The ascorbic acid concentrations were not affected by the different types of packaging tested. However, a decrease from 41.35 to 27.52 mg/g was observed during the 24 days of refrigerated storage

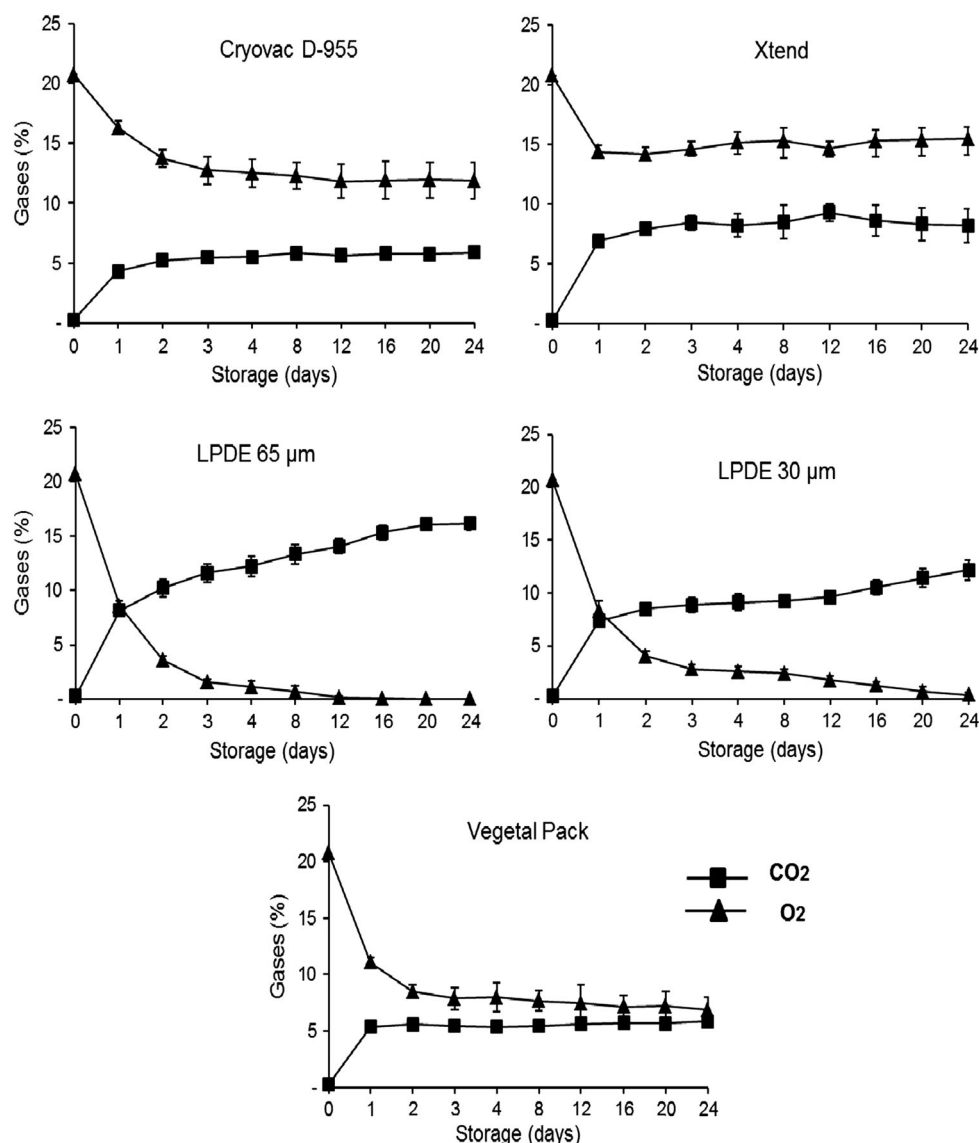


Figure 1. Gas composition of the atmosphere of packages containing 4.5 kg of 'Tahiti' acid limes that were wax coated and kept under refrigerated storage ($10 \pm 1^\circ\text{C}$; $75 \pm 5\%$ RH) for 24 days. The values are means \pm standard deviation. Vertical bars represent the standard error of the means.

followed by 6 days under ambient conditions. This decrease resulted from changes that occur during fruit maturation and senescence, characterized by the consumption of organic acids, namely ascorbic acid, in oxidation reactions.²³

A decrease in the initial acidity (6.09%) of the juice was observed following 30 days of storage, independently of the treatment. The fruits only coated with wax (control) presented the highest acidity (6.64%), followed by the fruits that were wax coated and packaged for 24 days in Vegetal Pack® (6.58%), Xtend® (6.56%), Cryovac® D-955 (6.46%), LDPE 30 µm (5.85%) and LDPE 65 µm (5.55%). The lower acidity of the limes packaged in LDPE bags, for both of the tested thicknesses, may be due to the high levels of CO₂, low levels of O₂ and the absence of equilibrated atmosphere inside the package.²⁴ In addition, these results indicate that this type of package had a negative effect on the fruit quality because in acid limes, a decrease in acidity is associated with senescence.

The treatments tested had no effect on the soluble solids concentration ($7.66 \pm 0.03^\circ\text{Brix}$). The minimum concentration of SS for commercialization of 'Tahiti' acid limes has been reported to be 6.5°

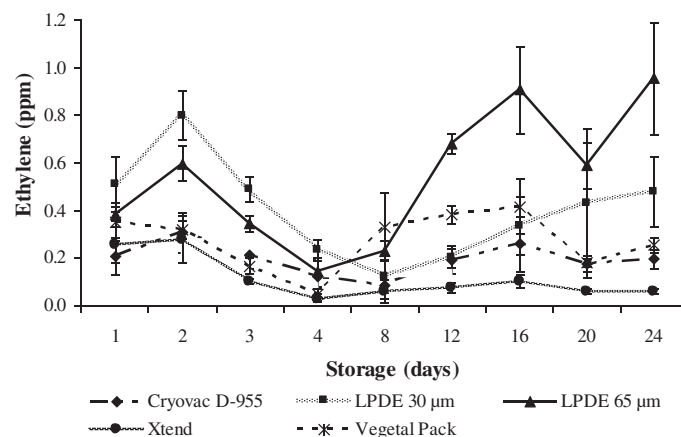


Figure 2. Ethylene concentration in the atmosphere of packages containing 4.5 kg of 'Tahiti' acid limes that were wax coated and kept under refrigerated storage ($10 \pm 1^\circ\text{C}$; $75 \pm 5\%$ RH) for 24 days. The values are averages \pm standard deviation.

Table 2. Total chlorophyll concentrations (mg/g) and peel color index of 'Tahiti' acid limes that were wax coated and packaged using different types of packaging then kept 24 days under cold storage ($10 \pm 1^\circ\text{C}$; $75 \pm 5\%$ RH) followed by 6 days under ambient conditions ($20 \pm 1^\circ\text{C}$; $75 \pm 5\%$ RH).

Packaging	Color index		Total chlorophyll	
	Storage (days)		Storage (days)	
	0	30	0	30
Control	-10.24 aA	-9.55 bc B	38.01 aA	18.94 aB
LDPE 30 µm	-10.24 aA	-10.72 aA	38.01 aA	18.79 aB
LDPE 65 µm	-10.24 aA	-10.29 abA	38.01 aA	19.17 aB
Vegetal Pack [®]	-10.24 aA	-9.62 bcB	38.01 aA	19.94 aB
Xtend [®]	-10.24 aA	-9.70 bcB	38.01 aA	19.84 aB
Cryovac [®] D-955	-10.24 aA	-9.54 cB	38.01 aA	17.11 aB

Averages followed by different lower-case letters within the same column and different upper-case letters within the same line are significantly different according to the Tukey test ($p < 0.05$).

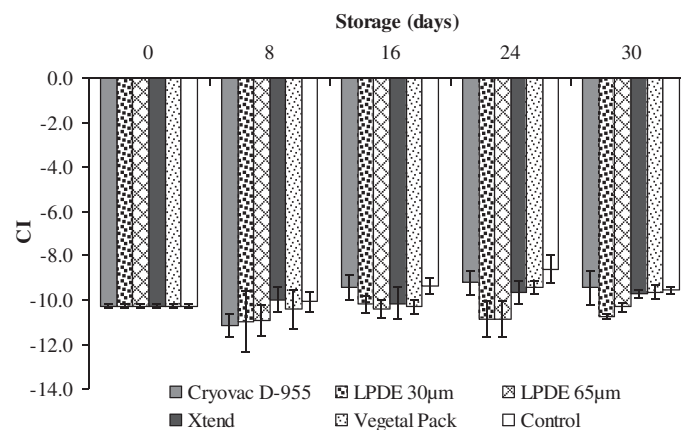


Figure 3. Peel color index of 'Tahiti' acid limes that were wax coated, packed with different types of packaging and stored at $10 \pm 1^\circ\text{C}$ for 24 days followed by 6 days at $20 \pm 1^\circ\text{C}$. The values are means \pm standard deviation.

Brix²⁵. It can therefore be inferred that although the different plastic films modified the atmosphere inside the packages in different ways, none of the tested treatments had a negative effect on the SS concentration that was large enough to classify the fruit outside commercialization standards. The application of polyethylene-based wax [Citrosol[®] (CITROSOL, Potries, Spain)] has been previously observed to not interfere in the soluble solid concentrations of acid limes stored at 10°C for 60 days followed by 3 days at 20°C².

The fruits that were only coated with wax (control) had lost more mass following 30 days of storage than the remaining treatments (Table 3). The combined use of wax and Cryovac[®] D-955, LDPE and Vegetal Pack[®] packaging was more efficient in preventing mass losses, maintaining a more turgid appearance and better commercialization conditions of the fruits, compared with Xtend[®]. This pattern may be due to the humidity-saturated environment inside the packages, resulting in decreased water vapor-pressure gradients between fruits and the atmosphere inside the package and consequently decreased transpiration, which is related to mass losses.²⁶ It should be noted that the combination of wax and the Xtend[®] plastic bags prevented mass losses, but in a less efficient way than the other packaging materials, perhaps due to the presence of micropores in the film.

The maximum loss of mass acceptable for citrus fruits is 5%²⁷. It can therefore be concluded that the limes packaged with the different types of packaging for 24 days, followed by 6 days without plastic film, were in conditions to be commercialized, whereas the ones that were only wax-coated were not suitable for commercialization. Other authors²⁸ also reported that the use of Carnauba wax on yellow passion fruits wrapped in Cryovac[®] D-955 film and stored in ambient conditions (20–25°C; 70–85% RH) for 12 days significantly decreased the loss of mass compared with using wax only.

The combined use of the wax coating and LDPE packages resulted in changes of the pulp color (Figure 4) and high acetaldehyde (1.48 and 1.66 g/100 g) and ethanol (202.78 and 283.63 g/100 g) juice concentrations. These patterns might have been a product of the low-oxygen levels in the atmosphere inside these packages (Figure 1), resulting in greater activity of enzymes such as pyruvate decarboxylase and alcohol dehydrogenase, which are responsible for anaerobic respiration and the production of fermentation metabolites, such as acetaldehyde and ethanol.²⁹ Furthermore, these fruits presented an alcohol odor. The presence of acetaldehyde and ethanol in citrus fruits is acceptable up to certain levels, but when present in high quantities, these compounds can result in flavor and/or aroma changes, decreasing fruit quality³⁰, as was observed in the present study.

The atmosphere modification with Xtend[®] packaging also resulted in the formation of acetaldehyde and ethanol in the juice (1.06 and 205.85 g/100 g, respectively), but these compounds were present in lower concentrations than for the limes packaged with LDPE. This result indicates that Xtend[®] also activated anaerobic metabolism, perhaps due to the low levels of O₂ (<4%) inside the package (Figure 1). Similar results were observed with Vegetal Pack[®] ($p > 0.05$), although the ethanol accumulation was lower (165.06 g/100 g), which may have been due to the O₂ and CO₂ inside the package being close to the desirable levels to maintain fruit quality (Figure 1).

The acetaldehyde and ethanol concentrations of limes packaged with Cryovac[®] D-955 were close to the levels detected at the beginning of the storage period (0.74±0.38 and 88.66±13.34 g/100 g, respectively) and close to the ones observed for fruits treated only with wax coating (0.54±0.10 and 81.01

Table 3. Loss of mass (%) of 'Tahiti' acid limes that were wax coated, packed with different types of packaging and stored at 10 ± 1°C for 24 days followed by 6 days at 20 ± 1°C.

Packaging	Loss of mass (%)
Control	6.33 A
Cryovac [®] D-955	3.45 C
LDPE 30 µm	2.85 C
LDPE 65 µm	2.98 C
Xtend [®]	4.39 B
Vegetal Pack [®]	2.98 C
C.V. (%)	8.09

The means followed by at least one common letter are not significantly different according to the Tukey test ($p < 0.05$).

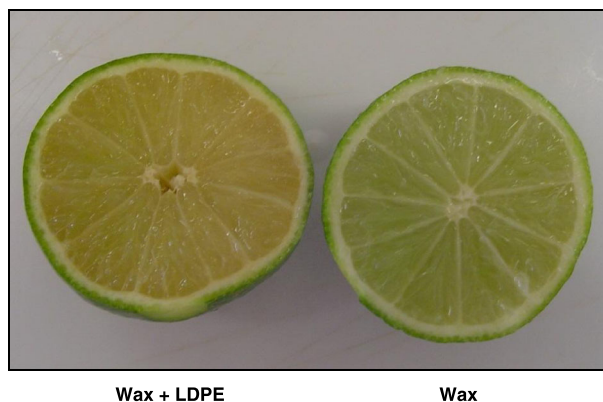


Figure 4. Appearance of the pulp of 'Tahiti' acid limes wax coated + packaged with LDPE plastic and those only wax coated (Control), stored for 24 days at $10 \pm 1^\circ\text{C}$ followed by 6 days at $20 \pm 1^\circ\text{C}$.

± 2.53 g/100 g, respectively). These results are in accordance with the data obtained for the modified atmosphere inside the packages, for which Cryovac® D-955 was different from the remaining packages tested in that it resulted in a modified atmosphere close to the desirable levels to maintain fruit quality. Acid limes have been reported to produce fermentation metabolites (ethanol, acetaldehyde and ethyl acetate) even under aerobic conditions,³⁰ which is in agreement with the present study.

CONCLUSIONS

The combined use of wax and plastic films Cryovac® D-955 and Vegetal Pack® resulted in better post-harvest preservation of 'Tahiti' acid limes than the isolated use of wax coating because the combined treatment maintained the green peel color following 30 days of storage (24 days under refrigeration + 6 days at ambient temperature) in addition to resulting in lower loss of mass and maintaining chemical characteristics adequate for fruit commercialization (SS = 6.5°Brix ; ascorbic acid = 20 to 40 mg/g; acidity = 6% citric acid). In addition, because the Cryovac® D-955 film is shrinkable, it makes the process of packaging at the packing house easier compared with Vegetal Pack® because the Cryovac® D-955 film relies on a mechanized, non-manual, process of packaging.

The associations of wax and LDPE packaging (30 and $65\ \mu\text{m}$) and Xtend® slowed the peel degreening but did not result in changes of the atmosphere to levels close to those desirable to maintain lime quality due to the accumulation of high concentrations of acetaldehyde and ethanol, especially for LDPE. LDPE packaging drastically changed the internal composition of packages, resulting in loss of the characteristic color of the pulp in addition to conferring an alcohol odor to fruits.

ACKNOWLEDGEMENTS

To the National Council for Scientific and Technological Development (CNPq) for the concession of a scholarship.

To the Foundation for Research Support of the State of São Paulo (FAPESP) for the financial support of the research project.

To the companies Cryovac Brasil Ltda, Stepac Brasil Ltda, and Eletropolímeros do Brasil Ltda for the supply of the plastic films.

To ABPEL, especially the Itacitrus company and its employees, for the supply of the 'Tahiti' acid limes.

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