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Effects of Packaging and Preservation Treatments on the Shelf Life of Murtilla Fruit (*Ugni molinae* Turcz) in Cold Storage

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Murtilla (*Ugni molinae* Turcz) fruit has a unique aroma; along with its pleasant sweet flavour, this has stimulated its commercial development in international markets. This development, however, requires the application of suitable conservation methods. Five different packaging treatments for murtilla fruit (Red Pearl-INIA variety) conservation were evaluated at 0 °C for 60 days. The treatments consisted of two types of packaging [polyethylene terephthalate (PET) punnets and low-density polyethylene (LDPE) bags] and the application of an edible coating of carboxymethyl cellulose to the fruits. The incorporation of the aqueous extracts of murtilla leaves from the 18-1 and 27-1 ecotypes into the carboxymethyl cellulose edible coating was also evaluated for its antimicrobial effects. There was a significant ($p < 0.05$) reduction in the moisture content, a significant weight loss and an increase in soluble solids when the murtilla fruit was packed only in a PET punnet. However, with the other four treatments, in which an LDPE bag and edible coating were used, the moisture content (76.8–74.4 g/100 g) and soluble solids (15.6–17.8 ºBrix) in the fruit remained stable during storage. Low weight loss (1.64–2.25%) occurred in the fruits that received treatments with the LDPE bag and edible coating. Under experimental conditions and from operational facilities, packaging in PET punnets with an LDPE bag was the best alternative to preserve the murtilla fruit at 0 °C for 60 days. Copyright © 2013 John Wiley & Sons, Ltd.

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INTRODUCTION

'Murtilla', 'mutilla' or 'murta' (*Ugni molinae* Turcz) is a native Chilean species belonging to the Myrtaceae family. The plant produces a small, round fruit with a diameter of 0.7 to 1.3 cm and a wide variety of colours.[1–3] This fruit has potential beneficial effects on human health and may

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be used as a preservative in food products.[4] Murtilla varieties are patented in the USA as Red and South Pearl-INIA.[5,6]

Murtilla fruits have a unique aroma and normally are obtained by collection from wild plants.[7,8] However, commercial murtilla plantations are being established for fresh fruit exporting. Packaging and refrigerated storage conditions are of great importance for the successful conservation of fruit quality. Some studies have evaluated the shelf life of fresh murtilla fruits for a maximum of 45 days.[9–11] However, so that the fruit quality can be maintained until it reaches consumers in international markets, an adequate packaging and refrigeration should be used to extend the shelf life to at least 60 days.

Murtilla fruits lose 9.7–17.5% of their weight after 45 days of storage in plastic boxes covered with a fine 'Europlas' plastic film at 4 °C and 80–85% relative humidity (RH).[9] Murtilla fruit weight decreased 21.7% after 15 days of storage (4 °C, 80–85% RH) when packed in plastic boxes covered with perforated cellophane paper.[10] Berger *et al.*[11] reported a high level of dehydration at 21 days (11%) and 42 days (18%) in the control samples of murtilla fruit packed in 100 g plastic boxes and stored at 1.5 °C under conventional air conditions. The weight loss in fruit due to dehydration in refrigerated storage has economic and quality implications. Consequently, control of the RH of the air surrounding the fruit in cold storage is necessary to avoid water loss.

Low-density polyethylene (LDPE) bags or films are normally used as packaging for international transportation of fresh fruits. The barrier properties of LDPE allow the generation of a modified atmosphere inside the bag to prevent dehydration.[12–15] The use of LDPE bags to preserve murtilla fruit quality has not yet been evaluated. Several recent studies have proposed the application of edible coatings (ECs) on fresh fruit to prolong the shelf life. The ECs provide different attributes, including a barrier effect against the flow of gases, structural resistance to water and microorganisms and sensory acceptability. The incorporation of bioactive compounds into EC can contribute to maintenance of fruit quality.[16,17] Murtilla leaf extracts have shown high levels of polyphenolic compounds with antioxidant, antimicrobial and barrier effect when incorporated into ECs.[18–21]

In the present study, different packaging conditions employing an LDPE bag and an EC with active extracts as a preservation method were evaluated for their effects on the shelf life of murtilla fruit (Red Pearl-INIA variety) in refrigerated storage at 0 °C for 60 days.

MATERIALS AND METHODS

Fruit, leaves and EC materials

Murtilla fruit of the Red Pearl-INIA variety was harvested from 3.5 year-old plants grown in the INIA-Carillanca experimental station, Puerto Saavedra, La Araucanía Region (38º45′S, 73º21′W), Chile. Fruits were transported to the laboratory immediately after harvesting. The murtilla fruit was harvested from plants chosen at random in a homogeneously farmed area with identical agronomic management, and it came from 10% of the total Red Pearl-INIA plants grown at the experimental station. Fruits that were immature, over-ripe or damaged were discarded. Murtilla leaves of ecotypes 18-1 and 27-1 were obtained from plants cultivated at the INIA station. Sodium carboxymethyl cellulose (CMC; 280–400 kDa, substitution degree 07–09) was purchased from Prinal S.A. (Santiago, Chile), glycerol (87%) from Merck (Darmstadt, Germany) and sunflower oil from a grocery store.

Packaging, preservation treatments and cold storage

Five treatments were evaluated, including two types of packaging and the application of ECs. The primary packaging used was a polyethylene terephthalate (PET) punnet (Typack S.A., Santiago, Chile); the internal dimensions were 93 × 93 × 30 mm, with 24 ventilation holes (4 × 10 mm) and a capacity of 125 g (4.4 oz). The secondary packaging was Ziploc® LDPE bags (17.8 × 20.3 cm, thickness 30 μm, S.C. Johnson and Son (Racine, Wisconsin, USA)) with a hermetic sealing system. LDPE oxygen and carbon dioxide permeabilities for 25 μm film thickness range from 4000 to 13 000 and 7700 to 77 000 cm^3/m^2 per 24 h/atm, respectively.[22] A value of 6510 cm^3/m^2 per 24 h/atm was reported for oxygen permeability of a Ziploc bag.[23]

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The EC applied to the fruit was CMC with glycerol and sunflower oil as plasticizing agents, as described by Bifani *et al.*[20] Two grammes of CMC was dispersed in distilled water (100 ml) and shaken in an incubator at 170 oscillations per minute at 25 °C for 24 h. The resulting solution was mixed by agitation (45 °C, 10 min). Glycerol (0.4 ml) and sunflower oil (0.5 ml) were added to CMC solution. In two treatments, the water was replaced by aqueous extracts of murtilla leaves of ecotypes 18-1 (EC18) and 27-1 (EC27), which had shown antioxidant activity and mechanical effects when added to fish gelatin film.[21] The aqueous extracts were obtained by placing the murtilla leaves (1.5 g) in distilled water (20 ml) at 25 °C for 10 min.[20] Before incorporation into the EC formulation, the total polyphenol content was adjusted to 207 mg/l measured by the Folin Ciocalteu method.[24] The fruits were coated by immersion in the EC emulsion (CMC, glycerol, oil and either water or aqueous extract) so that they were completely covered.

Approximately 100 g of fruit was packed in each replicate, and the samples were stored for 60 days at 0 °C (±0.2 °C) in a temperature-controlled chamber (Archiclima, Chile). During storage, the RH of the air inside the chamber fluctuated between 86% and 92%. The five treatments were identified as PET, PET–LDPE, EC–PET–LDPE, EC18–PET–LDPE and EC27–PET–LDPE. The oxygen and carbon dioxide levels inside the packaging were determined using PBI Dansensor 9900 equipment (Ringsted, Denmark) on days 1, 15, 30 and 60 (data not shown). The moisture content, weight loss, soluble solids (SS) and pH of the fruit were determined in three replicates on days 0, 15, 30 and 60. Mesophilic aerobic microorganisms on the fruit were determined on days 0, 30 and 60 (data not shown) according to the Chilean Health Public Institute methodology.[25]

Moisture content

Between 4 and 5 g of murtilla fruit was used to determine the moisture content. Fruits were cut in half, put in an oven at 105 °C for 2 h and then weighed. They were kept at 105 °C until a constant weight was reached.[26]

Weight loss

The weight loss of 100 g of fruit in three replicates for each treatment was monitored and recorded on days 15, 30 and 60 of storage using an analytical balance (±0.001 g).

Soluble solids and pH

Fifteen to twenty murtilla fruits were compressed, and the pulp was separated from the skin. The pulp was mixed and measured with a refractometer (Abbé). The pH was determined by introducing the electrode of a pH meter into the homogeneous mass. The pH meter was calibrated with buffered standards pH 4.01 and 7.00.

Statistical analysis

The data were subjected to factorial two-way analysis of variance (A × B), the sources of variance being treatments and days. Tukey's honestly significant difference test was used to determine significant differences between treatment means. Mean values were considered significantly different at $p < 0.05$. The data were analysed using the R language for statistical computing.[27]

RESULTS

Oxygen and carbon dioxide

The percentage of O_2 inside the packages was significantly reduced ($p < 0.05$) with only PET–LDPE treatment at day 60. There was a significant increase ($p < 0.05$) in the percentage of CO_2 when the fruits were packed with PET–LDPE, EC–PET–LDPE, EC18–PET–LDPE and EC27–PET–LDPE, showing a relationship between the O_2 consumed and the CO_2 produced when PET and LPDE packaging were used.

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Moisture content and weight loss

Table 1 shows that for the PET–LDPE, EC–PET–LDPE, EC18–PET–LDPE and EC27–PET–LDPE treatments, the Red Pearl-INIA murtilla fruit maintained constant levels of moisture during the 60 days at 0 °C, with average values varying from 76.8 to 74.4 g per 100 g fresh weight. Consistent with this result, it was also observed that a similar weight loss occurred in the fruits that received these treatments, with average losses fluctuating between 1.64% and 2.25% at the end of storage.

Soluble solids

Fresh murtilla fruit presented an average value of 16.8 ºBrix at the start of storage (Figure 1). For the PET treatment, the SS value for day 60 is not reported because it was not possible to obtain the pulp to determine this parameter owing to the dehydration of the fruit. The SS content in the PET treatment increased significantly ($p < 0.05$) over the storage period, whereas in the other treatments, it did not change significantly ($p > 0.05$), fluctuating between 15.6 and 17.8 ºBrix. These results show that the use of the LDPE bag and the application of the EC stabilize the SS.

Table 1. Moisture content and weight loss of fresh Red Pearl-INIA murtilla fruit stored at 0 °C for 60 days for the PET, PET–LDPE, EC–PET–LDPE, EC18–PET–LDPE and EC27–PET–LDPE treatments.

	Moisture content (g/100 g)				Weight loss (%)		
Treatment	Day 0	Day 15	Day 30	Day 60	Day 15	Day 30	Day 60
PET	76.8a	63.8b	42.5c	15.9d	31.76a	55.17b	71.54c
PET–LDPE	76.8a	76.8a	75.4a	75.4a	0.36d	0.88d	1.64d
EC–PET–LDPE	76.8a	76.3a	74.6a	74.4a	0.37d	1.00d	1.94d
EC18–PET–LDPE	76.8a	74.1a	74.5a	75.2a	0.63d	1.32d	2.25d
EC27–PET–LDPE	76.8a	73.8a	74.4a	74.8a	0.48d	1.10d	2.18d

The means and standard deviations were obtained from three replicate experiments. For the moisture content and weight loss, the different letters indicate a significant difference by Tukey's honestly significant difference test at $p < 0.05$.
PET, polyethylene terephthalate; LDPE, low-density polyethylene; EC, edible coating.

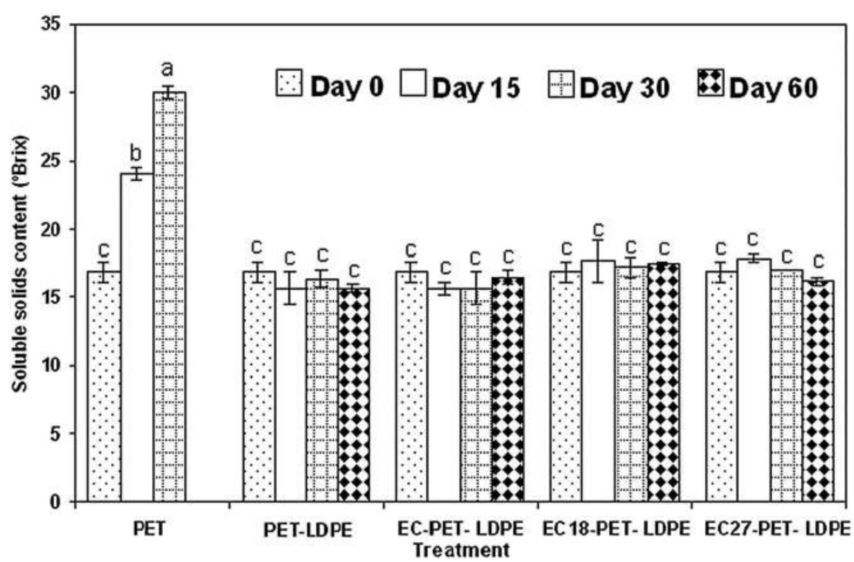

Figure 1. Soluble solids (°Brix) of fresh Red Pearl-INIA murtilla fruit stored at 0 °C for 60 days for five treatments. The means and standard deviations were obtained from three replicate experiments. Different letters indicate a significant difference by Tukey's honestly significant difference test at $p < 0.05$.

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pH

All of the treatments resulted in a significant increase ($p < 0.05$) of the pH during storage at 0 °C for 60 days (Figure 2). For the PET treatment, the pH value for day 60 is not reported for the same reason stated for SS.

Mesophilic aerobic microorganism count

Initially, murtilla fruit presented an average value of 3.2 log CFU/g of mesophilic aerobic microorganisms. All treatments were able to control the microbial growth because no significant variations ($p > 0.05$) were observed during storage.

DISCUSSION

Oxygen and carbon dioxide

The expected barrier effect of the LDPE bag allowed the increase in the proportion of carbon dioxide and the tendency to reduce the percentage of oxygen in the air surrounding the murtilla fruit in those treatments that used this bag. The beneficial effect of bag treatments shown in this experiment has also been reported for other fruits and types of packaging.[13,14,28,29]

Moisture content and weight loss

The PET–LDPE, EC–PET–LDPE, EC18–PET–LDPE and EC27–PET–LDPE treatments (Table 1) were able to maintain the initial condition to the same degree as the modified and controlled atmospheres used for the postharvest management of other fruits, such as strawberries (Honeoye and Korona cultivars) and blueberries, whose weight losses were at most 0.5% and negligible, respectively.[29,30] Murtilla leaf extracts incorporated in ECs have demonstrated effects on mechanical properties and decreased the water vapour permeability (WVP) of the CMC film.[20,21]

The application of a CMC EC to the fruits (EC–PET–LDPE treatment) and the incorporation of murtilla leaf extracts (EC18–PET–LDPE and EC27–PET–LDPE treatments) did not produce

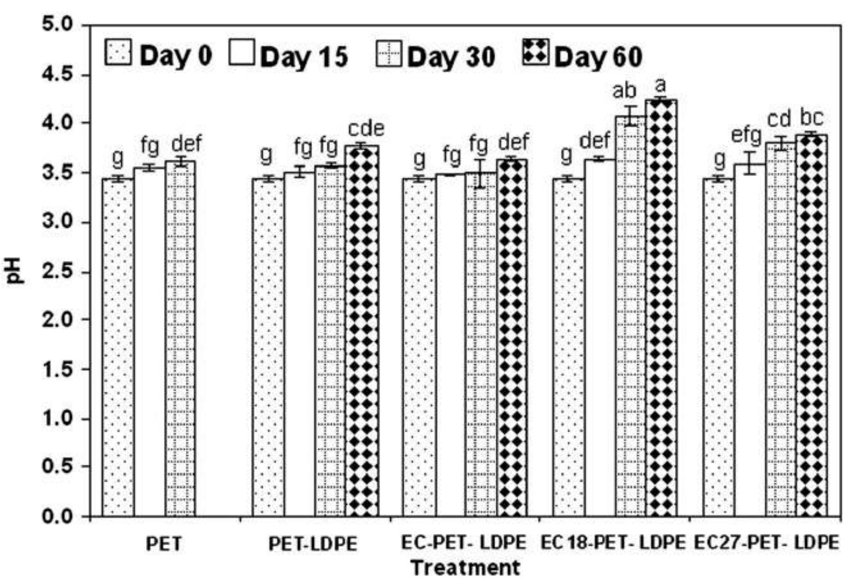

Figure 2. pH values of fresh Red Pearl-INIA murtilla fruit stored at 0 °C for 60 days for five treatments. The means and standard deviations were obtained from three replicate experiments. Different letters indicate a significant difference by Tukey's honestly significant difference test at $p < 0.05$.

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significant differences ($p > 0.05$) in the moisture content or weight loss when compared with the PET–LDPE treatment. This result suggests that the principal effect on the control of these two parameters is the LDPE bag. The low WVP of LDPE[22,31,32], whose values at typical conditions (38 °C, 90% RH) range from 7 to 9.7×10^{-13} g/m/s/Pa, probably allowed sufficiently high RH equilibrium to be reached inside the bags, which prevented fruit dehydration. The low WVP of LDPE would remain at 0 °C, and maybe its barrier effect has increased because the water vapour pressure at 0 °C is lower than at 38 °C. As expected, the LDPE bag is adequate to prevent the murtilla fruit dehydration, without requiring an additional barrier such as a CMC EC. The excessive reduction in the moisture content and high weight loss shown by the PET treatment was likely due to a lower RH in the chamber (86% to 92%), as the value required to reach equilibrium conditions is not less than 97% for most fresh fruits.[33]

Soluble solids

The SS content of the murtilla fruit on day 0 (Figure 1) agrees with the range (15.1 to 21.7 ºBrix) reported by Torres *et al*.[34] for wild murtilla fruit collected from Cauquenes (35º46′S) and Chiloé (42º34′S) in two seasons (1996/1997 and 1997/1998). Under modified and controlled atmosphere conditions, the SS in murtilla fruits were maintained without significant variation during 42 days at 1 °C.[11] In our experiment, the treatments that used the LDPE bags maintained stable SS for a period longer than those reported by Venegas *et al*.[10] and Berger *et al*.[11]

The effects of the three EC treatments were similar to the treatment without EC during storage at 0 °C for 60 days, suggesting that the PET–LDPE treatment proves to be the most suitable for controlling the moisture content, weight loss and SS under the conditions evaluated in this study (Table 1 and Figure 1). In the PET treatment, the significant increase in the SS may be attributed to the reduction of the moisture content, causing excessive dehydration. Berger *et al*.[11] also reported an increase in SS in murtilla fruit to a value of 30 ºBrix, which was attributed to dehydration in the control samples packaged in 100 g plastic punnets and stored in air at 1 °C.

pH

The initial pH (3.4) shown in Figure 2 is within the pH range (3.2 to 5.9) reported by Torres *et al*.[34] for wild murtilla fruit collected from Cauquenes to Chiloé in two seasons.

The use of the LDPE bag was not favourable for the pH, which changed in all treatments. Our results are not consistent with those reported by Berger *et al*.[11], who found no change in the pH of murtilla fruits during 42 days of storage. Only the PET–LDPE and EC–PET–LDPE treatments kept the pH stable until day 30 of storage. The greatest increase in murtilla fruit pH during the period evaluated occurred for the EC18–PET–LDPE treatment. In strawberries stored at low temperatures (0 and 6 °C), the pH either presented no significant variation or decreased because of the biosynthesis of acids such as citric acid.[35,36] However, strawberries stored for 10 days at 5 °C in various CO_2 concentrations showed a pronounced increase in its pH in the presence of higher concentrations of this gas.[37] In our study, the rise in the pH could be related to the significant increase in CO_2 for the four treatments in which the LDPE bags were used as secondary packaging.

Mesophilic aerobic microorganism count

A sanitary condition of murtilla fruit was evaluated by mesophilic aerobic microorganism showing counts below the limit set by the Chilean Health Ministry (5.7 log CFU/g).[38] The control of the microbial count during storage may be attributed to the presence of polyphenolic antimicrobial components, which have been identified in murtilla leaves and fruit.[19] Also, the temperature storage was not appropriate to mesophilic aerobic microorganism growth.

CONCLUSION

The use of an LDPE bag as a secondary package maintained the moisture content and SS of the Red Pearl-INIA murtilla fruit stored at 0 °C for 60 days. The mesophilic aerobic microorganism counts

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were not affected by the LDPE bags and ECs. Among the treatments evaluated, the use of PET punnets with an LDPE bag (PET–LDPE treatment) is the best alternative for the preservation of the murtilla fruit when kept at 0 °C for 60 days.

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