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Changes in the quality and antioxidant properties of fresh-cut melon treated with the biopreservative culture *Pseudomonas graminis* CPA-7 during refrigerated storage

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ABSTRACT

The strain CPA-7 of *Pseudomonas graminis*, isolated from apple by our group, was reported to reduce the population of foodborne pathogens in minimally processed fruit. The aim of this work was to investigate its effect on physical parameters, visual quality, enzymatic activity, vitamin C content and antioxidant activity of fresh-cut melon during refrigerated storage. CPA-7 treated or untreated fresh-cut melon was packaged under air or modified atmosphere (MAP) and stored at 5 and 10 °C for 8 days. Results showed a loss of texture during storage, above all at 10 °C. A good visual quality was observed for samples stored at 5 °C, although samples treated with the antagonistic strain CPA-7 and stored in air presented the lowest score, whereas those stored at 10 °C showed a poor visual appearance and a decrease of lightness. In general, CPA-7 treated samples presented higher POD values than untreated ones at 5 °C, and lower values at 10 °C. PPO increased during refrigerated storage regardless of the addition of the antagonist. Also, treatment with the antagonistic strain CPA-7 retained the antioxidant activity and vitamin C content of fresh-cut melon during refrigerated storage.

1. Introduction

Melon (*Cucumis melo* L.) is a widely consumed fruit in the world. One of the main cultivars present on Spanish markets is 'Piel de Sapo' due to its highly appreciated organoleptic attributes. Fresh-cut fruits are increasing their popularity in response to consumers demand for fresh-like, healthy and convenient products. Nevertheless, minimal processing promotes faster deterioration in relation to intact fruits. Tissues are damaged as result of operations such as peeling, coring, slicing or shredding, leading to an increase in respiration, biochemical changes and microbial spoilage. Sanitizers like chlorine along with modified atmospheres packaging and refrigerated temperatures are hurdles generally used to prolong the shelf life of minimally processed products (Rico et al., 2007). However, these treatments are unable to guarantee microbiological safety if foodborne pathogens, such as *Salmonella*, *Listeria monocytogenes* and *Escherichia coli* O157:H7, are present (Nguyen-the and Carlin, 1994; Ukuku and Sapers, 2007; Alegre et al., 2010a, 2010b; Abadias et al., 2012).

Biological preservation has emerged as a promising strategy to extend the shelf life and to improve the microbiological safety of foods (Schillinger et al., 1996; Gálvez et al., 2010). Several studies have been carried out on their application to fresh fruits (Conway et al., 2005; Trias et al., 2008; Fan et al., 2009; Alegre et al., 2012; Mari et al., 2012). Recently, our research group has isolated the strain CPA-7 of *Pseudomonas graminis* from apple, reporting that its application could reduce the population of foodborne pathogens in minimally processed apples, peaches and melon (Alegre et al., 2013a, 2013b; Abadias et al., 2014). Most of available studies in the literature have focused on microbiological aspects and a few of them on the activity of defence-related enzymes. In this context, Torres et al. (2011) demonstrated that the biocontrol agent *Pantoea agglomerans* CPA-2 triggered H_2O_2 production and superoxide dismutase (SOD) and catalase (CAT) enzymatic activities in oranges. Also, Lu et al. (2013) reported that postharvest treatment of citrus fruit with *Rhodosporidium paludigenum* enhanced the activities of defence-related enzymes including peroxidase (POD) and polyphenol oxidase (PPO). POD and PPO are important food quality-related enzymes that have been associated to changes in color, flavor, texture and nutritional quality. To our knowledge, no research has integrated the study of the effect of biological preservation on the quality, nutritional value and antioxidant properties of minimally

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processed fruits during refrigerated storage. Therefore, the objective of this work was to evaluate the effect of the antagonistic strain CPA-7 of *P. graminis* on the physical parameters, visual quality, enzymatic activities (POD and PPO), vitamin C content and antioxidant activity of minimally processed melon during storage at 5 and 10 °C.

2. Materials and methods

2.1. Plant material

'Piel de Sapo' melons (*C. melo* L.) were obtained from a local supermarket at commercial maturity. Fruits free of visual defects and uniform in color and size were selected. Melons were cleaned, sanitized by immersion into a 0.1 g/L sodium hypochlorite solution adjusted to pH 6.5 (Panreac, Spain), rinsed and dried prior to cutting operations.

2.2. Bacterial strain

The antagonistic strain of *P. graminis* CPA-7 was isolated from apple surface in our laboratory (Alegre et al., 2013a). CPA-7 strain was grown in tryptone soy broth (TSB, Oxoid, UK) for 20–24 h at 30 °C. For the inoculum preparation, bacterial concentration was estimated using spectrophotometer set at 420 nm according to standard curves, and a volume of the concentrated suspension was added to deionized water to obtain approximately 10^7 cfu (colony forming units)/mL. Inoculum concentration was checked by plating appropriate dilution onto Nutrient Agar (NA, Biokar Diagnostics, France), followed by incubation at 30 °C for 48 h.

2.3. Processing, packaging and storage

Melons were hand-peeled with a sharp knife and edible portions were sliced and then cut to obtain trapezoidal sections. Next, two groups of fruit were prepared: cut melon dipped (1:2 w/v) for 2 min at 4 °C in a 5 g/L calcium chloride (anhydrous, Panreac, Spain) solution (control) or in a 5 g/L calcium chloride solution plus CPA-7 (10^7 cfu/mL) (CPA-7). Treated fruit was allowed to dry at room conditions. Approximately 125 g of cut melon was placed in polypropylene trays (173 × 128 × 51 mm, ILPRA Systems España, S.L., Spain) and heat-sealed with a polypropylene film (64 μm of thickness, ILPRA) of low O_2 and CO_2 permeability [110 cm^3/(m^2 day atm) at 23 °C and 90% relative humidity]. Two atmospheres were studied, a passive modified atmosphere (MAP, using the film described previously) and air (Air, using the same film manually perforated with 9 holes of 400 μm each). Melon trays were stored at 5 °C and 10 °C for 8 days. At days 0 and 8, a portion of each sample was frozen with liquid nitrogen, pulverized and maintained at −80 °C for enzymatic assays, vitamin C analysis and antioxidant activity determination. Experiments at 5 °C and 10 °C were carried out independently. Initial quality parameters of melon batches are shown in Table 1.

2.4. Physical and physicochemical assays

Physical (color and texture) and physicochemical (pH, soluble solids and titratable acidity) assays were performed according to methods previously described (Abadias et al., 2014). Briefly, color was measured with a CR-200 Minolta Chroma Meter (Minolta, INC., Tokyo, Japan) using CIE L^*, a^*, b^* coordinates with illuminant D65 and 10° observer angle. L^* defines the lightness, and a^* and b^* define the red-greenness and blue-yellowness, respectively. The flesh color was also measured and expressed as the hue angle ($h° = \arctan(b^*/a^*)$). Texture evaluation was carried out by determination of the maximal strength necessary for a cylindrical probe of 4 mm of diameter and plane basis to penetrate 10 mm into a cylindrical sample of melon flesh of 20 mm height, using a TA-TX2 Texture Analyser (Stable Micro Systems Ltd., Surrey, England). pH was determined using a penetration electrode in a pH-meter model GLP22 (Crison Instruments SA, Barcelona, Spain). Soluble solids were measured at 20 °C with a hand-held refractometer (Atago Co. Ltd., Tokio, Japan) in juice extracted by crushing melon pieces in a blender. To measure titratable acidity, 10 mL of melon juice were diluted with 10 mL distilled water and it was titrated with 0.1 N NaOH up to pH 8.2.

Table 1
Initial quality parameters of melon[a].

Parameters	Batch 1 (5 °C)	Batch 2 (10 °C)
Acidity (g citric acid/L)	1.21 ± 0.08 a	1.27 ± 0.11 a
pH	5.93 ± 0.07 a	5.93 ± 0.07 a
Soluble solids (%)	10.7 ± 0.1 b	11.0 ± 0.1 a
Texture (*N*)	4.38 ± 0.18 a	3.57 ± 0.97 a
L^*	60.44 ± 3.47 a	60.51 ± 3.76 a
a^*	-3.17 ± 0.73 a	-4.27 ± 1.55 a
b^*	9.83 ± 1.96 a	11.33 ± 3.12 a
POD (ΔOD/min/mg prot)	9.89 ± 0.67 a	13.54 ± 2.35 a
PPO (ΔOD/min/mg prot)	0.76 ± 0.12 a	0.51 ± 0.09 b
Ascorbic acid (mg/100 g fw[b])	9.33 ± 0.90a	10.93 ± 0.99 a
Vitamin C (mg/100 g fw)	11.19 ± 1.64 a	13.57 ± 2.64 a
Antioxidant activity		
DPPH• (μmol ascorbic acid/100 g fw)	74.66 ± 6.72 a	91.57 ± 15.16 a
FRAP (μmol ascorbic acid/100 g fw)	107.83 ± 16.11 a	114.43 ± 13.70 a

[a] Values are the mean of independent determinations ± standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$).
[b] fw, fresh weight.

2.5. Assessment of overall visual quality

The visual quality was determined by an untrained panel based on the following hedonic scale: 9 = excellent; 7 = very good; 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible (Altisent et al., 2014).

2.6. Peroxidase (POD) and polyphenol oxidase (PPO) assays

POD and PPO activities were determined according to the method of Jang and Moon (2011) with minor modifications (Altisent et al., 2014).

2.6.1. POD activity

The POD activity was determined spectrophotometrically at 475 nm. POD activity was expressed as units of enzyme per milligram of protein being one unit defined as an absorbance increase of 0.001 per min.

2.6.2. PPO activity

The PPO activity was determined spectrophotometrically at 505 nm. PPO activity was expressed as units of enzyme per milligram of protein being one unit defined as an absorbance increase of 0.001 per min.

2.7. Protein determination

Protein concentration of enzymatic extracts was determined employing a Bio-Rad kit for the Bradford reaction at 595 nm, with bovine serum albumin as standard.

2.8. Determination of vitamin C

The procedure employed to determine total vitamin C was the reduction of dehydroascorbic acid to ascorbic acid, using