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Characterization of microbial population of breba and main crops (*Ficus carica*) during cold storage: Influence of passive modified atmospheres (MAP) and antimicrobial extract application

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ABSTRACT

The purpose of this work was to study the changes of bacterial and fungal population of breba fruits such as 'Banane' and 'San Antonio' as well as 'Cuello Dama Negro', 'Cuello Dama Blanco' and 'San Antonio' fig cultivars stored in passive modified atmospheres (MAP) by the use of three different microperforated films (M10 with 16 holes; M30 with five holes and M50 with three holes). Moreover the effects of the application of aqueous soy polyphenolic antimicrobial extract (APE), alone or combined with MAP, were also studied for 'Cuello Dama Negro' and 'Cuello Dama Blanco' fig cultivars. Bacteria and fungi isolates were identified by PCR-RFLP of 16S rRNA and ITS regions, respectively, and subsequently sequence of the different patterns obtained. The results indicated that *Pseudomonas gessardii*, *Pantoea agglomerans* and *Enterobacter asburiae* were the main species of bacteria found in all the treatments studied. The fungal species identified were *Aureobasidium pulullans*, *Cladosporium cladosporioides* and *Alternaria alternata*, which were found in a lower percentage in fruit stored in MAP and fruits treated with antimicrobial extracts, as this treatments allowed to reduce the microbial growth of moulds and yeasts. Thus, the application of treatments such as M30, M50 or the combination of MAP with antimicrobial extract was highly effective to control fruit spoilage in fig and breba crops.

1. Introduction

Breba and fig crop (*Ficus carica* L.) are native to central Asia, although they have been spread throughout the Mediterranean region where it is well-adapted to several types of soils and climates. Fig trees produce one or two crops per year, depending on the cultivar. Breba crop fruits are borne on old shoots and are larger and juicier than the main crop figs, which is produced by the axils of leaves (Pereira et al., 2015). Both breba and fig crop are climacteric fruit classified as a kind of syconium. These fruits are an important part of the Mediterranean diet, being appreciated for their sensorial and nutritional characteristics, what has increased consumer interest in fresh breba and fig crop. Nevertheless, breba and fig crop are distinguished by their high perishability, so that increasing global demand for products and services call for new postharvest techniques in order to extend the shelf life of these fruits (Kong et al., 2013).

The main postharvest losses in these fruits are caused by the growth and proliferation of microorganisms. The predominant microflora of fresh breba and fig is mostly composed of moulds (Crisosto et al., 2011) such as *Botrytis cinerea, Monilinia laxa, Alternaria alternata, Fusarium moniliforme, Rhizopus stolonifer, Aspergilus niger, Cladosporium herbarum,* and *Phytophora palmivora*, being most of them responsible of fruit decay.

Among the main diseases reported in breba and fig fruits we can find: endosepsis caused by *Fusarium* spp. or smut caused in dried fruit by *Aspergillus niger*, Alternaria rot (caused by *Alternaria alternata* or other *Alternaria* spp) (Cantín et al., 2011; Crisosto et al., 2011). Additionally, these moulds can be pathogenic or toxigenic

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due to their mycotoxins production hability while growing on fruits (Cantín et al., 2011), even during its cold storage (Tournas and Stack, 2001) Likewise, yeast such as *Hanseniaspora* spp., and *Torulopsia* spp have been reported as the main yeast species in fruits (Chand-Goyal and Spotts, 1996a; Tournas and Katsoudas, 2005; Van der Steen et al., 2002). Specifically, species belonging the genus *Hanseniaspora, Saccharomyces, Pichia* or even bacteria such as *Bacillus* may cause souring or fermentation (Cantín et al., 2011).

Other microbial groups such as mesophilic aerobic, lactic acid bacteria (LAB), *Staphylococcus* spp., *Enterobacteriaceae* spp., *Pseudomonas* spp. and *Acetobacter* spp. have been described in a lesser extent as part of the microbial population in ripe fruits and vegetables, which can be also responsible for fruit decay and health hazard (Badosa et al., 2008).

The use of packaging under modified atmosphere (MAP) has been reported to reduce the respiration rate, with the benefit of delaying senescence, not only by reducing metabolic activity, but also microbial proliferation (Jacxsens et al., 2001, 2002; Saltveit, 2003; Valero et al., 2008). These effects of MAP have been previously reported by Villalobos et al. for fresh breba fruits (2014) and figs (2016), who reported that the use of microperforated films to create passive modified atmospheres was effective in reducing the incidence of decay caused by fungal growth and in retaining quality characteristics. Thus, atmospheres with low levels of O_2 and high levels of CO_2 inhibit the growth of most aerobic microorganisms due to the well known antimicrobial activity of CO_2 at high concentration (Babic and Watada, 1996; Devlieghere et al., 2000; Jacxsens et al., 2001; Serradilla et al., 2013). Other technologies such as the application of biocontrol agents and natural compounds such as essential oils or aqueous phenolic extracts have been used in food preservation due to their antimicrobial properties (Isman, 2000; Korsten et al., 1991; Janisiewicz et al., 2001). These compounds inhibit the mycelial growth of moulds and spore germination, affecting the cellular metabolism of the pathogens (Regnier et al., 2010; Serrano et al., 2005; Tzortzakis, 2007a, 2007b). Moreover, the combination of both technologies renders them more effective to prolonging the postharvest life as well as the safety of the horticultural produce (Serrano et al., 2008; Villalobos et al., 2015). However there are no published data about the influence of postharvest technologies such as modified atmospheres or antimicrobial treatments in the microbiological population of fig and breba crops.

Thus, the aim of the present study was to investigate the microbiological changes during cold storage under different modified atmospheres (MAP) and the application of natural antimicrobial soy extract alone and in combination with MAP for different fig and breba crop cultivars in order to develop a system for maintaining microbiological quality and extend postharvest life.

2. Materials and methods

2.1. Plant material

Different fig and breba cultivars were used. Regarding breba fruits, the cultivars employed were 'San Antonio' (SA) and 'Banane' (BN) (Villalobos et al., 2014), while for figs, the cultivars selected were 'San Antonio' (SA), 'Cuello Dama Blanco' (CDB) and 'Cuello Dama Negro' (CDN) (Villalobos et al., 2016) All fruits were harvested (handpicked) at the commercial ripening stage according to their firmness and skin colour, early in the morning from 6-year-old fig trees from an experimental orchard at an altitude of 223 m above sea level in the research centre Finca La Orden-Valdesequera (latitude 38° 85′ 2015) 19″ N, longitude −6° 68′ 28″ W, Guadajira, Badajoz, Spain and immediately transferred to the laboratory under cold conditions.

For the development of the antimicrobial postharvest treatment, soybean flour that was kindly supplied by ACOREX company (Extremadura, Spain). Soybean four was used to get an aqueous soy polyphenolic antimicrobial extract (APE). In order to extract and purify the phenolic extracts in the soybean flour, 5 g of flour were extracted with 30 mL ethanol-water-hydrochloric acid (80:19:1 v/v). The extracts were separated from the solids by filtration with Whatman No 1 filter paper and concentrated under reduced pressure in a rotary evaporator at 37 °C. Thus, the final APE obtained presented a total phenolic concentration of 0.859 mg of gallic acid equivalents per gram and was main composed of with isoflavones and phenolic acids such as syringic acid, as described Villalobos et al. (2015).

2.2. Experimental procedure

For the experimental design of this study, two different postharvest techniques were assessed:

2.2.1. Breba and fig fruits packaged under MAP

Two breba fruit cultivars, 'Banane' and 'San Antonio' and three different fig cultivars, 'Cuello Dama Negro' (CDN), 'Cuello Dama Blanco' (CDB) and 'San Antonio' (SA), were selected for the development of this study due to their good aptitude for fresh consumption and high acceptability. For each cultivar, ten fig fruits and eight breba fruit (approximately 400 g) homogeneous in colour and size and without visual defects were selected and packaged in polypropylene (PP) punnets (26 × 16 cm, 416 cm^2) and sealed with different microperforated 40-μm thick biaxially oriented polypropylene (BOPP) films obtained from ACSA Films (Valencia, Spain) under atmospheric conditions. A total of 192 punnets of breba fruits and 288 punnets for fig fruits were classified in different batches for each cultivar as follows (Table 1):

- Control batch (C): Six punnets for each sampling day (0, 7, 14, 17 and 21) and per cultivar were packaged with macroperforated film with five holes (ø = 9 mm)
- M10 batch: Six punnets for each sampling day and per cultivar were packaged with microperforated film with one hole per 10 mm (a total of 16 holes, ø = 100 μm)
- M30 batch: Six punnets for each sampling day and per cultivar were packaged with microperforated film with one hole per 30 mm (a total of five holes, ø = 100 μm)
- M50 batch: Six punnets for each sampling day and per cultivar were packaged with microperforated film with one hole per 50 mm (a total of three holes, ø = 100 μm)

2.2.2. Application of aqueous soy phenolic extract (APE) alone and in combination with MAP in fig cultivars

For the study of the application of APE only two fig cultivars, 'Cuello Dama Blanco' (CDB) and 'Cuello Dama Negro' (CDN), were selected, since 'San Antonio' fig cultivar was not suited for this kind of treatment due to the easily damaged skin of this cultivar and the wide opening of the ostiole.

Previously to the experiment, the phenolic extract was adjusted at pH 4.5 with NaOH 1 N and phenolic concentration at 1 g/L with sterile distilled water by the Folin-Ciocalteu colorimetric method according to Lima et al. (2005). The application of the extract was carried out by dipping both fig cultivars for 30 s at a refrigeration temperature of 7 °C. Then, the figs were dried at room temperature. Finally, ten fruits (approximately 400 g) of each cultivar were packaged in polypropylene (PP) punnets (26 × 16 cm, 416 cm^2), as previously described. A total of 96 punnets for fig fruits were classified in different batches for each cultivar as follows (Table 1):