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Efficacy of natural antimicrobials to prolong the shelf-life of minimally processed apples packaged in modified atmosphere

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ABSTRACT

Minimally processed fruit are susceptible to microbial proliferation and to a fast loss of sensory quality. In this experimental work, in order to increase shelf-life and quality parameters (texture and colour) maintenance of sliced apples (*Malus communis*, var. *Golden delicious*), the use of natural antimicrobials was proposed as alternative to the traditional sanitization methods. Citron EO, hexanal, 2-(*E*)-hexenal, citral and carvacrol, alone or in combination, were employed. As control, apples washed only with 0.5% of ascorbic and 1% of citric acid were used. The apples were dipped with traditional or natural antimicrobial solutions according to a defined protocol. After the treatment, apples were packaged in active modified atmosphere (7% O_2 and 0% CO_2), into medium permeability bags. The products were stored at 6 °C and, immediately after washing and during storage, the yeast cell loads were monitored until the spoilage threshold (6 log CFU/g). In addition, the volatile profiles, electronic nose analyses, colour and texture analyses were monitored during the storage. In all the samples the spoilage yeast threshold was not attained within the 35 days of storage independently on the substance or mixture of substances supplemented. Samples treated with the combinations citral/2-(*E*)-hexenal and hexanal/2-(*E*)-hexenal showed a good retention of colour parameter during storage. Among investigated samples, hexanal/2-(*E*)-hexenal treatment promoted the best retention of firmness throughout 35 days of storage. These results evidence the potentiality of dipping treatment based on these natural antimicrobials to strongly prolong the shelf-life of fresh-cut apples.

1. Introduction

Minimally processed fresh fruit represent an important component of a healthy diet and are a convenient way of increasing fresh produce consumption. Minimally processed fruit are susceptible to microbial proliferation due to the loss of natural resistance and their high water and nutrient content (Brackett, 1994; Rico, Martín-Diana, Barat, & Barry-Ryan, 2007). In addition, the raw materials during processing are subjected to peeling, cutting or slicing that favour the microbial growth due to the release of nutrient and the transport of the surface microbiota on the cut surfaces (Lanciotti et al., 2003; Rojas-Grau et al., 2007). The absence of treatments able to guarantee the microbial stability, the active metabolism of fruit tissue, and the confinement of final product inside the packaging increases the growth potential of the naturally occurring microorganisms (Lanciotti et al., 2003; Nguygen & Carlin, 1994). Due to the lack of processing steps or factors able to kill microbial contaminants, an efficient temperature control during manufacture, distribution and retailing is required for maintaining the microbiological quality and the safety of these products. However, the maintaining of the cold chain and the use of chemicals as disinfectants of raw materials are not sufficient to either eliminate or significantly delay the microbial spoilage of these products entirely and to ensure the product safety (Soliva-Fortuny & Martín-Belloso, 2003). In fact, a wide literature shows the presence on fresh fruit and related minimally processed products of

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pathogenic species such as *Listeria monocytogenes*, *Salmonella* spp., *Yersinia enterocolitica*, *Aeromonas hydrophila* and *Staphylococcus aureus* (Alegre, Abadias, Anguera, Oliveira, & Vinas, 2010; Beuchat, 1998; Conway et al., 2000; Gunes & Hotchkiss, 2002). Moreover, fresh fruit, fruit juices and minimally processed fruit have been incriminated in several outbreaks caused by *Escherichia coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* (Abadias, Alegre, Usall, Torres, & Vinas, 2011; Harris et al., 2003; Olaimat & Holley, 2012; Powell & Luedtke, 2000; Van Boxstael et al., 2013).

Currently, several investigations have been focused on the search for natural antimicrobials able to increase the quality and safety of the minimally processed fruit (Allende, Selma, López-Gálvez, Villaescusa, & Gil, 2008; Beuchat, 1998; De Azeredo et al., 2011; López-Gálvez, Allende, Selma, & Gil, 2009; Vandekinderen, Devlieghere, De Meulenaer, Ragaert, & Van Camp, 2009). A wide literature shows the great potential as antimicrobials in model and food systems of essential oils from citrus fruit (Espina et al., 2011; Fisher & Phillips, 2008; Settanni et al., 2012). Moreover, the action of single constituents of these oils has been studied to identify their cell targets and the most active molecules, and to balance their intrinsic variability (Karatzas, Bennik, Smid, & Kets, 2000; Kurekci et al., 2013; Picone et al., 2013; Sado Kamdem, Belletti, Magnani, Lanciotti, & Gardini, 2011; Vazquez, Fente, Franco, Vazquez, & Cepeda, 2001; Zheng, Bae, Joung, Heu, & Lee, 2013). In particular, citral (3,7-dimethyl-2-7-octadienal), is a terpenoid with 2 isomers, geranial and neral, naturally occurring in citrus essential oils and characterized by a wide spectrum antimicrobial activity both in model and real foods (Belda-Galbis, Pina-Peréz, Leufvén, Martinéz, & Rodrigo, 2013; Hayes & Markovic, 2002; Wuryatmo, Klieber, & Scott, 2003). Citral and citron essential oil at concentration compatibles with sensorial features were able to significantly prolong the microbial shelf-life of the fruit-based salads in syrup (Belletti, Lanciotti, Patrignani, & Gardini, 2008), and the stability of fruit based soft drink (Belletti et al., 2007).

Also the antimicrobial activity of hexanal and 2-(*E*)-hexenal, which are components of the aroma of many fruit and vegetables, has been already tested in model (Gardini, Lanciotti, Caccioni, & Guerzoni, 1997; Gardini, Lanciotti, & Guerzoni, 2001; Kubo & Fujita, 2001) as well as in real systems (Corbo, Lanciotti, Gardini, Sinigaglia, & Guerzoni, 2000; Lanciotti et al., 2003, 2004; Lanciotti, Corbo, Gardini, Sinigaglia, & Guerzoni, 1999). Hexanal, 2-(*E*)-hexenal, and hexyl acetate improved shelf-life and safety of minimally processed fruit (Lanciotti et al., 2004; Serrano et al., 2008). In particular, the addition of hexanal and 2-(*E*)-hexenal in storage atmosphere of fresh-cut apples resulted in a positive effect on shelf-life, due to their antimicrobial activity against naturally occurring spoilage species also when deliberately inoculated at levels of 10^3 CFU/g. Moreover, these molecules determined the enhancement of the sensorial properties, as well as the retention of the original colour of the packaged products (Corbo et al., 2000; Lanciotti et al., 1999). These aldehydes showed a great potential as antimicrobials also against pathogens such as *Salmonella* spp., *E. coli* and *Pseudomonas aeruginosa* (Kubo & Fujita, 2001). Little information is available on the relationship between the outgrowth of spoilage microorganisms, their volatilome, and the perception of the decay of minimally processed vegetables by consumers.

In this perspective, the main aim of the work was to study the effects of citron EO, hexanal, 2-(*E*)-hexenal, citral and carvacrol alone or in mixture on the shelf-life of minimally processed apples packaged in modified atmosphere. In particular, the effects of these aroma compounds on yeast and lactic acid bacteria (LAB) cell loads, texture, colour and volatile molecule profiles were monitored during the storage at 6 °C. An additional aim of this work was the identification of eventual spoilage volatile markers in relation to the natural antimicrobial used.

2. Material and methods

2.1. Natural antimicrobials

The tested compounds (hexanal, 2-(*E*)-hexenal, citral and carvacrol) were purchased from Sigma–Aldrich (Milano, Italy). Citron essential oil (EO) was obtained from Flora s.r.l. (Pisa, Italy). Citron EO and the natural tested antimicrobials were selected both for their antimicrobial activity and impact on organoleptic properties after a preliminary screening. Citron EO was preliminarily characterized by GC/MS-SPME technique to know the exactly composition of the oil (Belletti et al., 2008).

2.2. Preparation of sliced apple products

Apples (*Golden delicious* sp.) were purchased at a local retailer in the same day of the sample preparation, were washed with running water at 13 °C for 2 min and then dried with blotting paper. After that, apples were peeled and sliced into cubes of roughly 15 cm^3. The citron EO and natural antimicrobials were added to apples with the dipping (1% citric acid + 0.5% ascorbic acid). Eight different treatment solutions at a temperature of 13 °C were prepared with running water. Two contained only citral or hexanal (250 mg/L); the others were mixtures of citral/hexanal (125/125 mg/L), citral/2-(*E*)-hexenal (125/125 mg/L), hexanal/2-(*E*)-hexenal (125/125 mg/L), citral/citron EO (125/125 mg/L), citron EO/carvacrol (200/50 mg/L). Natural antimicrobials were conveyed through 1% (v/v) of ethanol. Control apple slices were subjected to dipping treatment without the supplementation of natural antimicrobials. Prepared apples were immersed and gently agitated into appropriate treatment solution for 2 min and with a ratio apples/water of 1:5 (w/v). After the treatment, apples were dried with paper and packaged in active modified atmosphere with 7% O_2 and 0% CO_2, into 59 μm-thick bags (CO_2 permeability at 22 °C: 2720 cm^3/m^2/day, O_2 permeability at 22 °C: 986 cm^3/m^2/day) with 35 g of product and a ratio apples/headspace of 1:1. Apples were stored at 6 °C until the end of shelf-life.

2.3. Microbiological analyses

During storage, the evolution over time of LAB and yeasts was evaluated by plate counting respectively on de Man Rogosa and Sharpe Agar (MRS, Oxoid Ltd. Basingstoke, Hampshire, United Kingdom) with added 0.05% cycloheximide (Sigma–Aldrich) and Sabouraud Dextrose Agar (SAB, Oxoid Ltd.), added to 0.02% chloramphenicol (Sigma–Aldrich), respectively. Microbiological analyses were performed after homogenization of the samples following their decimal dilution in physiological solution (10 g of sample diluted into 90 mL of physiological water (0.9% (w/v) NaCl)). For the detection of the natural occurring *L. monocytogenes*, the method suggested by McClain and Lee (1988) was followed, whereas the occurrence of *Salmonella* spp. was investigated according to the method proposed by Andrews and Hammack (1998). *E. coli* was investigated on violet red bile agar (Oxoid) added to 4-methylumbelliferyl-β-D-glucuronide (Oxoid), incubating the plates at 37 °C for 24 h. The potential *S. aureus* was enumerated on Baird-Parker media (Oxoid) with added egg yolk tellurite emulsion (Oxoid) after 24 h at 37 °C. The analyses were performed immediately after treatments and after 3, 7, 10, 12, 14, 17, 21, 24, 28, 31 and 35 days of storage.

2.4. Volatile molecule profiles and electronic nose analyses

Apple packages were used for headspace volatile compound analysis by GC/MS-SPME technique. For each treatment condition the samples were analysed immediately after the treatments and