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Shelf-life extension of fresh-cut "Fuji" apples at different ripeness stages using natural substances

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Abstract

Shelf-life extension of fresh-cut "Fuji" apple at two stages of ripeness (partially ripe and ripe) using natural substances was evaluated. Cylinders of fresh-cut "Fuji" apples were immersed for 1 min in: (a) an aqueous solution of *N*-acetyl-L-cysteine at 1% (w/v), glutathione at 1% (w/v) and calcium lactate at 1% (w/v) (CGLW), (b) CGLW and D-L-malic acid at 2.5% (w/v) (CGLW + MA) or (c) sterile distilled water (W). Gas production, firmness, color and behavior of native flora (mesophilic bacteria, psychrophilic bacteria, yeasts and mould) were studied weekly over 30 days. Sensory evaluation of fresh-cut apples was carried out at 0 and 15 days during storage. Statistically significant differences ($p < 0.05$) were found in ethylene and ethanol production between fresh-cut partially ripe and ripe apples, respectively, reaching values of ethylene of 8.92 and 134.11 $\mu L\,L^{-1}$ and ethanol of 28.73 and 59.39 $\mu L\,L^{-1}$ at 30 days of storage. The firmness of fresh-cut apples was significantly different ($p < 0.05$) between partially ripe (initial values of 12.4 ± 0.8 N) and ripe apples (initial values of 8.3 ± 1.0 N), but did not differ throughout the storage time, nor among dipping conditions. An important reduction in lightness ($p < 0.05$) was observed throughout storage in the fresh-cut apples with all dipping conditions. However, an influence of the ripeness stage on microbiological stability was not detected. A reduction in growth rate and an increase in the lag phase of mesophilic and psychrophilic bacteria, yeasts and moulds were found in fresh-cut apples dipped in CGLW + MA, which gave as a result an extension of 13 days over the microbiological stability of fresh-cut apples immersed in W (10.1 days). Thus, the use of a combination of CGLW + MA might be a good low cost alternative for the fresh-cut industry since it can offer better maintenance of the physicochemical characteristics and microbiological stability of fresh-cut apples, ensuring a shelf-life of, at least, 14 days.

Keywords: Apple; Shelf-life; Ripeness; Malic acid; *N*-Acetyl-L-cysteine; Glutathione; Calcium lactate

1. Introduction

In recent years a rapid expansion in the sale of prepacked/precut fresh fruit and vegetables has been taking place because of the advantages offered by those commodities to consumers, such as freshness, convenience and low calorific content. However, as a result of the active metabolism of the plant tissues, and damage during peeling, grating, shredding and the exposure of their cut surfaces to external factors, the produce changes from having a relative stability with a shelf-life of several weeks or months to a perishable product with a very short shelf-life (Ahvenainen, 1996; Lanciotti et al., 1999). For those reasons, achieving microbiologically safe products with sensory and nutritional fresh-like characteristics is still a challenge, in spite of the research efforts already made.

The deterioration of lightly processed fruit is a complex process concerning both physicochemical and biochemical modifications and microbial spoilage. Injury stress caused by processing results in cellular decompartmentalization or delocalization of enzymes and substrates which leads to various types of biochemical deterioration such as browning, off-flavors, and texture breakdown. Moreover, peeling and cutting facilitate primary infection of the plant tissues by epiphytic and phytopathogenic microorganisms (Varoquaux and Wiley, 1994).

Enzymes and substrates are normally located in different cellular compartments and their transfer is actively regulated. Processing results in destruction of surface cells and injury stress of underlying tissues. Enzymatic reactions cause sensory

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deterioration such as off-flavor, discoloration, and loss of firmness (Varoquaux and Wiley, 1994). Enzymatic browning of minimally processed apples is a major problem affecting processing of the fruit, polyphenol oxidase being the most important enzyme involved. Enzymatic browning requires the presence of four components: oxygen, an oxidizing enzyme, copper and a suitable substrate. To prevent browning, at least one of these components must be removed from the system (Ahvenainen, 1996). Browning and its control have been extensively studied and reported in foods including fruit and vegetables (Iyengar and McEvily, 1992; McEvily and Iyengar, 1992; Ahvenainen, 1996; Friedman, 1996; Laurila et al., 1998). Traditionally, sulphites have been used to prevent browning in fruit and vegetables; however, their use can have negative health effects, such as with asthma (Ahvenainen, 1996). Therefore, various approaches to control the extent of browning have been tried, such as the use of restricted O_2 atmosphere conditions with low-temperature storage to prevent enzymatic browning of apple pieces. However, this approach is not sufficient to attain browning stability because the high phenolic content of the fruit diminishes the effectiveness of elevated CO_2 atmospheres in preventing browning of fresh-cut apples; it has been concluded that reduction of O_2 levels to negligible levels is necessary to inhibit polyphenol oxidase (PPO) mediated browning of many fresh-cut fruit products (Buta et al., 1999). Other approaches proposed are the use of browning inhibitors with several different types of biochemical functions alone or in combination with modified atmospheres and low-temperature storage. Reducing agents such as citric acid, ascorbic acid, isoascorbic acid, sodium erythorbate and 4-hexylresorcinol (Ahvenainen, 1996; Sapers and Miller, 1998; Buta et al., 1999; Dong et al., 2000; Soliva-Fortuny et al., 2001, 2002), as well as sulphydryl (SH)-containing amino acids and peptides such as cysteine and glutathione (Molnar-Perl and Friedman, 1990; Richard et al., 1991; Richard-Forget et al., 1992; Buta et al., 1999; Gorny et al., 2002; Rojas-Graü et al., 2006; Oms-Oliu et al., 2006), have been used to prevent the browning of fresh-cut apples, pears and potatoes. Calcium salts, particularly calcium chloride and lactate, are used generally in combination with those browning inhibitors as firmness agents in a wide variety of whole, peeled, and fresh-cut fruit and vegetables.

Minimal processing may increase microbial spoilage of fruit through transferring skin microflora to fruit flesh, where microorganisms can grow rapidly upon exposure to nutrients (Corbo et al., 2004). Fungi and mesophilic bacteria are the principal flora present on fruit, mainly because of their pH. However, the incidence of other microorganisms as parasites, viruses and food-borne pathogenic bacteria, such as *Listeria monocytogenes*, *Salmonella* serovars and some *Escherichia coli* strains, has also been reported (Harris et al., 2003; Lanciotti et al., 2003; Eswaranandam et al., 2004). In fact, the number of documented outbreaks of human infections associated with consumption of raw and minimally processed fruit and vegetables has considerably increased during the past decades (Lanciotti et al., 2003).

The use of natural antimicrobial compounds, such as organic acids, is a good practise for the food industry because they can prevent microorganism growth and additionally avoid browning. The main acid present in apple is malic and its concentration varies widely among apples varieties (Eisele and Drake, 2005).

The main objective of this study was to extend the shelf-life of fresh-cut "Fuji" apples at different ripeness stages using *N*-acetyl-L-cysteine, glutathione, calcium lactate and D-L-malic acid.

2. Materials and methods

2.1. Materials

"Fuji" apples (*Malus domestica* Borkh), partially ripe (commercial ripeness stage) and ripe (advanced ripeness stage) apples were provided by ACTEL, Lleida, Spain. *N*-acetyl-L-cysteine with 98% degree purity (Acros Organics, New Jersey, USA) and reduced glutathione with 98% degree purity (Acros Organics, New Jersey, USA) were used as antibrowning substances, calcium lactate pent-hydrate, extra pure (Scharlau Chemie S.A., Barcelona, Spain) was applied as an anti-softening agent and D-L-malic acid, extra pure (Scharlau Chemie S.A., Barcelona, Spain), was used as an antimicrobial.

2.2. Flesh apple characterization

Characterization of the apples was made following the official methods for fruit juices and other vegetables and derivatives (BOE, 1988). Titrable acidity, pH (Crison 2001 pH-meter; Crison Instruments S.A., Barcelona, Spain), soluble solids (%) content (Atago RX-1000 refractometer; Atago Company Ltd., Japan), color (Minolta CR-400 Chroma Meter; Konica Minolta Sensing Inc. Osaka, Japan) and firmness (TA-TX2 Texture Analyzer; Stable Micro Systems Ltd., Surrey, England) were the evaluated parameters (Table 1).

2.3. Dipping solutions

Two aqueous solutions of *N*-acetyl-L-cysteine at 1% (w/v), reduced glutathione at 1% (w/v), calcium lactate pent-hydrate at 1% (w/v) in sterile distilled water (CGLW), and *N*-acetyl-L-cysteine at 1% (w/v), reduced glutathione at 1% (w/v), calcium

Table 1
Physicochemical properties of fresh-cut partially ripe and ripe "Fuji" apple

Parameters	Partially ripe	Ripe
pH	4.20 ± 0.01[a]	4.37 ± 0.01[a]
Soluble solids (%)	12.4 ± 0.1[a]	13.50 ± 0.17[a]
Total acidity (malic acid g/100 ml)	0.421 ± 0.013[a]	0.233 ± 0.006[a]
Color		
*L**	75.0 ± 1.1[b]	72.53 ± 0.30[b]
*a**	−1.40 ± 0.27[b]	−1.96 ± 0.23[b]
*b**	22.9 ± 1.1[b]	22.5 ± 0.5[b]
Firmness (N)	13.8 ± 1.3[b]	7.2 ± 0.6[b]

[a] Mean of three analysis ± standard deviation.
[b] Mean of 10 analysis ± standard deviation.