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Impact of different calcium dips and solution pH on quality of ready-to-eat baby-leaf spinach

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ABSTRACT

The effect of calcium application and solution pH on ready-to-eat (RTE) baby-leaf spinach (*Spinacia oleracea* L.) texture and structure preservation was studied. Spinach leaves were treated with calcium chloride, calcium lactate and calcium propionate, at two different pH conditions (5 and 7), packaged and stored for 7 days at 5 °C. After 24 h, the leaves crispness increased 49 and 29% for leaves treated with calcium chloride and lactate at pH 5 and the elasticity increased 100% after calcium propionate treatment at pH 7. During spinach shelf-life, the tissue flexibility decreased (20–60%) for all calcium treatments at pH 5 and 7 while tissue crispiness increased (7–40%) only for calcium treatments under pH 7. The electrolyte leakage increased throughout storage and was higher for all calcium treated samples when compared with controls. The chlorophyll content slightly decreased (12%) by the end of product shelf-life and did not differ according the calcium treatments.

Total vitamin C content was lower in leaves treated with calcium chloride at pH 5 (31%) and pH 7 (19%) while the remaining treatments did not affect vitamin C content.

The different calcium additives tested for potential texture quality maintenance did not provide the expected benefits on baby spinach leaves but increasing solution pH from 5 to 7 lead to an increased firmness by the end of shelf-life.

1. Introduction

Visual appearance and texture are two fundamental quality parameters determining the acceptability of processed produce (Toivonen and Brummell, 2008). As farming methods, handling chain, washing equipment and packaging become highly optimized to handle the leaves without causing excessive damage, further improvement must focus on leaf properties and postharvest quality (Rico et al., 2007a).

During mechanical operations, leaf surfaces are damaged, exposing and releasing enzymes which then spread through the tissue and come into contact with their substrates (Oms-Oliu et al., 2010). The enzyme pectinmethylesterase (PME) demethylates pectin resulting in a pectin molecule with a lower degree of methylation. After that, pectin can undergo depolymerization as a result of the polygalacturonase (PG) action, which breaks down –1,4 glycosidic bonds in pectin, leading to cell wall degradation (Alandes et al., 2006). Additionally, calcium can interact with the free carboxyl groups released by the de-esterification of pectin promoted by PME. From this esterification, insoluble calcium pectates are formed, which strengthen the structure of the cell wall (Mignani et al., 1995; Wehr et al., 2004; Toivonen and Brummell, 2008). Therefore, in order to avoid texture loss and to preserve the structure, minimally processed products can be treated with calcium salts such as chloride, lactate, ascorbate and propionate (Lamikanra and Watson, 2004; Oms-Oliu et al., 2010).

These water-soluble calcium salts (ascorbate, chloride, lactate, propionate, and gluconate), and even the less soluble calcium carbonate, have been successfully used to reduce softening of fresh-cut fruit and vegetables (Oms-Oliu et al., 2010). Calcium lactate has been widely used for delicate fruit with a high senescence index, such as grapefruit (Baker, 1993), peaches (Manganaris et al., 2007), fresh-cut cantaloupes (Lamikanra and Watson, 2004) and apples (Anino et al., 2006).

Calcium application often results in improved cell-wall structures (Alandes et al., 2006), and reduced incidence of physiological disorders and decay (García et al., 1996). There has been interest in the use of calcium solutions for extending the

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shelf-life of fresh-cut vegetables (Garcia and Barrett, 2002). However, no reports have been found on the calcium effect on spinach texture quality maintenance. Most of the studies involving spinach report the effect of storage conditions, such as modified atmosphere (Gil et al., 1999), storage temperature (Bergquist et al., 2006) and industrial processing (Hodges and Toivonen, 2008) on quality. The latest study, describes that washing, drying and packaging affected spinach quality during storage, showing that hydrocooling is an important step in the preservation of membrane integrity and that drying and packaging steps are the one that imposed higher mechanical damage to membranes and reduction of chlorophyll content (Hodges and Toivonen, 2008).

The majority of works on vegetables have described an increase in crispiness as a result of calcium application, namely calcium lactate on fresh-cut lettuce (Martín-Diana et al., 2006) and carrots (Martín-Diana et al., 2005).

The main purpose of this study was to determine the effect of different calcium solutions, at two different pH values (5 and 7) on physiological and nutritional quality of baby spinach leaves, during refrigerated storage (7 days).

2. Material and methods

2.1. Plant material

Spinach (*Spinacia oleracea* L.) leaves were cultivated under commercial conditions at Odemira (South-west Region, Portugal) by Vitacress Portugal SA. The soil had a particle size distribution of 40% sand, 36% silt and 24% clay, a soil texture classified as loam (USDA textural soil classification, 1987). The harvest was carried out mechanically, during summer, in the early morning (6 am) and immediately after harvest, baby spinach leaves were cooled down to 5 °C, under vacuum chamber and transported under refrigerated conditions to the CBQF-UCP laboratory (Porto, Portugal) with maximum transport time of 5 h.

2.2. Calcium solutions

Three water-soluble calcium salts – chloride, lactate and propionate – were dissolved to a final concentration of 250 mM in order to obtain 1% calcium (Ca^{2+}) solutions. To evaluate the effect of pH *per se* in calcium solutions, pH value was adjusted to 5 and 7 using citric acid and sodium hydroxide, respectively.

2.3. Washing and storage conditions

Freshly harvested spinach leaves, at commercial maturity, were transported as described previously and immediately after arrival, leaves were inspected for uniformity and visual defects, washed in cold water and dipped in 100 μg. L^{-1} sodium hypochlorite solution, for 1 min. After washing and decontamination, spinach leaves were dipped separately in the three calcium solutions, at different pH values (5 and 7) for 3 min, at 10 °C. Calcium propionate at pH 5 formed precipitates, therefore it was not used in this experiment. The leaves dipped only in hypochlorite solution were used as controls. Excess solution was removed by centrifugation for 30 s, in a salad spinner. Then, processed leaves (250 g) were manually packaged in polypropylene microperforated bags (300 cm × 360 cm, Belca S.A., Guipúzcoa, Spain) with a film oxygen transmission rate (OTR) of 20,500 cm^3. linear m^{-1} day^{-1} atm^{-1}, heat sealed with a packaging machine (Clatronic FS 3261), stored in the dark for 7 days at 5 ± 0.5 °C and assessed (at day 1, 4, 5, 6 and 7) for quality as function of the interaction between calcium salts and solution pH. All the experiments were performed in triplicate.

2.4. Firmness assessment

The parameters of spinach leaf texture were determined using a heavy duty platform (HDP/90) with a 5 mm stainless steel spherical probe (P/5S) and using a 5 kg load cell attached to a TA.XT2i Texture Analyzer (Stable Micro Systems, Godalming, U.K.). Pre-test speed was set at 2 mms^{-1}, test speed was 1 mms^{-1}, and post speed was 10 mms^{-1}. For each analysis, one spinach leaf was used, and compressed to 10 mm. Force and time data were recorded using an Exponent Stable Microsystem Texture Expert (Version 6.1.8.0) from Stable Micro Systems Ltd. Software. From the force-versus-distance curve, values for the following textural parameters were calculated: burst strength and distance to burst during compression. Twenty measures for each replicated sample were performed at each experimental condition tested.

2.5. Electrolyte leakage

Electrolyte leakage as an estimation of cell membrane integrity (Fan and Sokorai, 2005), was determined, according to the recommendations of Saltveit (2002). Randomly selected leaves were cut into discs of 22 mm, with the help of a cork-borer. Leaves discs (5 g) were incubated at 23 °C in 100 mL plastic bottles containing 40 mL of deionized water. During incubation, samples were agitated at a speed of 120 rpm using a shaker. Electrical conductivity of the bathing solution was measured at 1 min (C_0) and 240 min (C_{4h}) of incubation, using a HANNA HI9813-6 conductivity meter (Italy). The samples were then slowly frozen at −18 °C for 16 h, and after slowly defrosting the bathing solution, the total conductivity (C_T) was measured. Three replicates per treatment were performed. Electrolyte leakage (E) was calculated from the following equation 1:

$$\boldsymbol{E} = \frac{(\boldsymbol{C_{4h}} - \boldsymbol{C_0})}{\boldsymbol{C_T}} \times 100 \quad (1)$$

2.6. Chlorophyll content

Leaf chlorophyll content was determined using leaf readings from a portable chlorophyll meter (Konica Minolta SPAD-502 Plus; Minolta, Osaka, Japan). The SPAD-502 measurements were performed on each analysis day with 12 readings for each replicated package, from each calcium treatment. The adaxial side of the leaves was always placed toward the emitting window of the instrument and major veins were avoided.

2.7. Vitamin C determination

Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined at 24 h and 7 d after storage through high performance liquid chromatography with diode array detector (HPLC-DAD) as described by Zapata and Dufour (1992). The determination of total vitamin C content (AA + DHAA) was achieved after derivatization of DHAA into the fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-*b*]quinoxaline-1-one (DFQ), with 1,2-phenylenediamine dihydrochloride (OPDA). The samples were analyzed with a HPLC-DAD (Waters Series 600, Massachussets, USA). Separation was performed in a reverse phase Symmetry® C18 column (250 × 4.6 mm i.d. 5 μm particle size and 125 Å pore size) with a guard column containing the same stationary phase (Symmetry® C18).

The mobile phase was methanol:water (5:95, v/v) containing 5 mM cetrimide and 50 mM potassium dihydrogen phosphate, at pH 4.5. The flow rate was 1.0 mL min^{-1}. The detector wavelength was set to 348 nm for DHAA and 261 nm for AA detection. Compounds retention times and spectra were analyzed by