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# Antioxidant responses in minimally processed celery during refrigerated storage

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#### Abstract

This work studies the effect of storage temperature (0, 4 and 10 °C) and time on the antioxidant capacity of cut celery packaged in polystyrene trays sealed with PVC film. Samples were taken at 0, 7, 14, 21 and 28 days of storage to determine total phenols, chlorogenic acid and ascorbic acid. The browning potential and antioxidant capacity of the product were also evaluated. The antioxidant power presented similar behaviour for the three temperatures tested, decreasing after the first 7 days and then increasing up to day 14. Such increase coincided with an elevation of the ascorbic acid content, which was stronger for higher temperatures. As a general conclusion, minimally processed celery retained its initial antioxidant capacity for a period of 21 days at 0 °C, showing the lowest levels of browning potential at this temperature.

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1. Introduction

During minimal processing, fruits and vegetables are treated in a series of stages where their structure and tissues are generally damaged or removed. By cutting, the size of diverse organs is reduced to obtain ready-to-use products that are packaged in small portions for convenience. During handling, cutting, washing and rinsing, important mechanical damage occurs, which is accompanied by oxidative stress. Disinfection by immersion in chlorinated water is still widely used for simplicity and low cost, though it constitutes an additional damaging factor because of hypochlorous acid reactivity (Wei, Cook, & Kirk, 1985).

The synthesis of several phenylpropanoid compounds (flavonoids, isoflavonoids, psoralens, coumarins, pheno-

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lics acids, chlorogenic acid, lignin and suberin) is induced in plants by biotic and abiotic stress, factors such as wounding, low temperature and attack of pathogens (Dixon & Paiva, 1995). In vegetables, responses can be oxidation of preformed phenolic compounds, synthesis of monomeric phenols and production of polymeric phenolic compounds (Rhodes & Wooltorton, 1978). Some notable consequences of these mechanisms are enzymatic browning and lignification of growing tissues, which damage various minimally processed products. Besides, the antioxidant capacity of this food group may be affected, with important consequences on nutritional quality.

Phenolic compounds are known to constitute one of the most important groups of natural antioxidants, owing to their diversity and extensive distribution. They possess biological and chemical properties in common: reducing character, capacity of sequestering reactive oxygen species (ROS) and several electrophiles, for chelating metallic ions, tendency to self-oxidation and

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capacity for modulating the activity of some cell enzymes (Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999).

Functions of diverse phenolic antioxidant in the diet have been discussed in many works (Astley, 2003; Block, Patterson, & Subar, 1992; Gutteridge, 1993; Hertog & Hollman, 1996; Kinsella, Frankel, German, & Kanner, 1993; Trewavas & Stewart, 2003). The biological activity of phenylpropanoids and their function as antimicrobial agents are well recognised, as are their antiallergenic and anti-inflammatory properties, along with their antimutagenic action (Rice-Evans, Miller, & Paganga, 1996).

On the other hand, ascorbic acid is an antioxidant that directly or indirectly sequesters harmful free radicals, usually present in live cells. For instance, it plays an essential role in capturing hydrogen peroxide and protects thiol groups of enzymes and proteins from oxidation (Foyer, 1993). According to McCarthy and Matthews (1994), minimal processing of fruits and vegetables may reduce ascorbic acid content of tissues. By contrast, other authors have reported that ascorbate synthesis is increased under stress conditions, and that modifications in the ascorbate pool would provide a good index of the stress experienced by a vegetable tissue (Stegmann, Schuler, Ruff, Knollmüller, & Loreth, 1991).

Celery is a plant material that easily adapts to minimal processing and constitutes an important regional crop. According to information provided by the Horticultural Census of the Great Buenos Aires Green Belt, its annual production in 1998, carried out under cover, reached 4400 t in this region.

The aim of the present work on minimally processed celery was to evaluate the effect of cutting, storage temperature and time on the contents of substances with antioxidant activity, such as ascorbic acid, total phenols and chlorogenic acid.

#### 2. Materials and methods

#### 2.1. Plant material, processing and storage conditions

Celery plants (*Apium graveolens* L.) cv Golden Boy, grown in a greenhouse, were received from a La Plata grower (Province of Buenos Aires, Argentina). Golden Boy is a white or self-whitening variety, widely cultivated in the zone. Two months after being transplanted, and once reaching commercial size, plants were harvested early in the morning, brought to the laboratory, and processed immediately.

Leaves and 4-cm long segments of the basal rosette were eliminated to obtain unbranched petioles. They were washed in running drinking water to remove any soil residues, and subsequently cut with a sharpened knife in 4-cm long strips. These were disinfected by immersion in chlorinated water (100 ppm active chlorine, pH 6–6.5, 8 °C) for 3 min and rinsed in a manual domestic centrifuge. Finally, the material was packaged in polystyrene trays (15 × 10 × 5 cm³), and covered with self-adhering PVC film (thickness, 10  $\mu$ m; O<sub>2</sub> permeability, 11,232 cm³ m<sup>-2</sup> atm<sup>-1</sup> day<sup>-1</sup>; CO<sub>2</sub> permeability, 48,552 cm³ m<sup>-2</sup> atm<sup>-1</sup> day<sup>-1</sup>; water vapour permeability, 40 g m<sup>-2</sup> day<sup>-1</sup>).

Trays containing about 175 g of product were kept for 28 days in cold stores at 0, 4 and 10 °C with a relative humidity of 85%. Samples (three trays for each time–temperature combination) were taken for analysis at 0, 7, 14, 21 and 28 days. Storage experiments were done in triplicate. Since the results were very similar for different conditions, here we provide those corresponding to one of them.

#### 2.2. Determinations

#### 2.2.1. Sampling

For each combination of time and temperature, the material from three trays was combined and homogenised. Part of the pool was frozen with liquid  $N_2$  and crushed in a mill (Janke and Kunkel Ika Labortechnik A10, Staufen, Germany). From this material, exactly weighed subsamples were taken in order to carry out the corresponding determinations.

### 2.2.2. Browning potential

Ten grammes of tissue frozen in liquid  $N_2$  and crushed as described above were treated with ethanol 96° for 60 min and then centrifuged at 11,500g, 10 °C for 30 min, retaining the supernatants. A further amount of ethanol was added to complete a final volume of 25 ml. Absorbance at 320 nm was measured on an aliquot of this extract. Extractions and determinations were carried out in duplicate, and the final results were expressed as absorbance units (AU)  $g^{-1}$  fresh tissue.

# 2.2.3. Total phenols content

Extraction was conducted as described in Section 2.2.2. Aliquots (20 ml) of the extracts were concentrated at reduced pressure (30 mm Hg, 40 °C) in a rotary evaporator R-124 (Büchi Labortechnik AG, Flawil, Switzerland), to dryness. Residues were resuspended in doubly distilled water. Total phenols were quantified employing the Folin–Ciocalteu reagent (Swain & Hillis, 1959). Absorbance readings were carried out at 760 nm. Duplicated extractions and determinations were conducted. Catechin was used as standard in a 3.75–12.75  $\mu$ g ml<sup>-1</sup> concentration range. Final results were expressed as  $\mu$ mol g<sup>-1</sup> fresh tissue.

# 2.2.4. Chlorogenic acid concentration

Extraction was conducted as described in Section 2.2.2. Aliquots of the extracts (20 ml) were concentrated

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