Available online at www.sciencedirect.com
ScienceDirect
LWT 41 (2008) 919–924
LWT
www.elsevier.com/locate/lwt

Phenylalanine ammonia lyase activity in fresh cut lettuce subjected to the combined action of heat mild shocks and chemical additives

S.I. Roura[a,b,*], L. Pereyra[a], C.E. del Valle[a]

[a]Grupo de Investigación en Ingeniería en Alimentos, Facultad de Ingeniería, Universidad Nacional de Mar del Plata, Juan B. Justo 4302, CP 7600, Mar del Plata, Argentina
[b]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

Received 11 August 2006; received in revised form 6 June 2007; accepted 14 June 2007

Abstract

Enzymatic browning reduces the visual quality of lettuce. In producing minimally processed lettuce, the stresses caused by wounding associated to cutting operations enhance enzymatic activity. Mild heat shocks with chlorinated water with calcium ions or ascorbic acid were assayed as a mean of reducing phenylalanine ammonia lyase (PAL) activity. Heat shocks reduced four times the rate of change in PAL activity during the first hours of storage over the rate for samples not subjected to thermal treatment. The addition of ascorbic acid resulted in an increase in PAL activity over samples where only chlorinated water was used. When calcium ions were introduced in the thermal baths, the PAL activity was reduced about four times, especially when the cutting operation was performed after the thermal shocks. Short heat shocks is a physical preservation technology that could be applied in countries with low technological development. This technology was efficient to control vegetable browning by reducing PAL activity.

Keywords: Romaine lettuce; Enzymatic browning; Mild heat shocks; Pal activity

1. Introduction

Lettuce is a leafy vegetable which is difficult to process because of its high mechanical and physiological fragility. One of the most common postharvest disorders of lettuce is the browning of cut pieces (Saltveit, 2000). Altered phenolic metabolism is involved in lettuce tissue browning. Wounding during the preparation of fresh cut lettuce induces the synthesis of enzymes of phenylpropanoid metabolism, the synthesis and accumulation of phenolic compounds and subsequent tissue browning. The first step in the phenylpropanoid pathway is the conversion of the amino acid L-phenylalanine to *trans*-cinnamic acid by the enzyme phenylalanine ammonia lyase (PAL; EC 4.3.1.5). Subsequent reactions produce several new compounds such as 5-caffeoylquinic acid (chlorogenic acid), 3,5-dicaffeoylquinic acid, caffeoyltartaric acid and dicaffeoyltartaric acid that have been related to browning in cut lettuce (Tomás-Barberán, Gil-Muñoz, Castañer, Artés, & Saltveit, 1997; Tomás-Barberán, Loaiza-Velarde, Bonfanti, & Saltveit, 1997). Both wounding and exposure to the plant hormone ethylene stimulate the phenylpropanoid pathway and induce new enzymatic activity leading to increased production of the major phenolic compounds and the synthesis of new compounds (Ke & Saltveit, 1989; Tomás-Barberán, Loaiza-Velarde, et al., 1997). The intensity of damage in the production of minimally processed cut Romaine lettuce directly affects the physiological response of the samples and the rate of change in PAL activity during the first day of storage (Pereyra, Roura, & del Valle, 2005). As browning greatly reduces the visual quality of cut lettuce it is important to prevent browning reactions. The techniques used to retard browning include low storage temperatures (Bolin & Huxsoll, 1991), modified or controlled atmospheres (MA or CA) with low oxygen and/or high carbon dioxide concentrations (Heimdal, Kuhn, Poll, & Larsen, 1995; Mateos, Ke, Cantwell, & Kader, 1993), chemical additives (McEvily, Iyengar, & Otwell, 1992) or the use of

*Corresponding author. Grupo de Investigación en Ingeniería en Alimentos, Facultad de Ingeniería, Universidad Nacional de Mar del Plata, Juan B. Justo 4302, CP 7600, Mar del Plata, Argentina.
E-mail address: sroura@fi.mdp.edu.ar (S.I. Roura).

0023-6438/$34.00
doi:10.1016/j.lwt.2007.06.005

brief heat shocks (Loaiza-Velarde, Mangrich, Campos-Varga, & Saltveit, 2003; Saltveit, 1998, 2000).

Several chemical compounds are reported to have inhibitory effects on enzymatic browning of various commodities. Sapers, Garzarella, and Pilizota (1990) reduced darkening of potatoes by using a dip treatment with ascorbic acid, $CaCl_2$ and citric acid. Calcium has an important role in maintaining quality of fruits and vegetables in respect to structural integrity of membranes and cell walls (Poovaiah, 1986). Calcium binds anionic groups of all membranes to form bridges between structural components, thereby maintaining cell permeability and compartmentation and structural integrity (Conway, Sams, McGuire, & Kelman, 1992). Izumi and Watada (1994, 1995) reported the effects of calcium treatments on the shelf life extension of fruit and vegetables. These effects are reduced respiration, suppression of ethylene production, increased firmness retention and reduced incidence of physiological disorders and decay. Dip treatment with ascorbic acid has retards enzymatic browning in freshly prepared vegetables (Roura, Moreira, Ponce, & del Valle, 2003). Heat treatments are effective as a non-chemical means of improving postharvest quality for a variety of horticultural products. A brief heat shock (90 s at 45 °C) disrupts the wound-induced increase in PAL activity, delaying and diminishing the accumulation of phenolic compounds and tissue browning (Loaiza-Velarde, Tomás-Barberán, & Saltveit, 1997). Murata, Tanaka, Minoura, and Homma (2004) showed that the heat shock treatment is useful for prolonging the shelf life of cut lettuce, repressing the induction of PAL activity and phenolic accumulation during storage, and preventing tissue browning. Chlorine is widely used to reduce postharvest decay of fruits and vegetables as a disinfectant. Water containing from 50 to 200 mg l^{-1} of chlorine with a contact time of 1–2 min is widely used to sanitize whole vegetables as well as fresh cut produce on a commercial scale (Klaiber, Baur, Wolf, Hammes, & Carle, 2005).

In a previous work, Roura et al. (2003) analyzed the effects of dip treatments with chlorine, citric acid, ascorbic acid and $CaCl_2$ on the storage life of Romaine lettuce leaves. The cooperative union of polygalacturonate chains with calcium ions makes the cell walls less accessible to enzymes that cause tissue softening or to cell wall-degrading enzymes (Conway, Sams, Wang, & Abbott, 1994). In this way, the addition of calcium salts preventing enzyme binding to specific substrate could reduce browning reactions. Ascorbic acid is a water-soluble antioxidant that acts as a reducing agent for several oxidant reagents reducing the rate of browning in fruits. Although PAL reaction is not oxidative and therefore ascorbic acid, as an antioxidant, would not exert a direct effect it could nevertheless act on the phenolic compounds produced by PAL activity.

Roura et al. (2003) indicated that although the presence of chlorine delayed degradation related to the activity of the native and exogenous microflora, the other additives (citric acid, ascorbic acid and $CaCl_2$) did not improve the storage life of lettuce when used alone. Bolin, Stafford, King, and Huxsoll (1977) working with shredded lettuce and Krahn (1977) with cut lettuce reported that the use of calcium treatment did not extend the storage life of the product. Izumi and Watada (1994) indicated that the lack of response to calcium by lettuce compared to other products might be due to differences in attributes of the product, its form, concentration of solution and storage temperature. Bolin and Huxsoll (1991) found that dipping salad-cut lettuce in 0.5 g/100 g ascorbic acid increased the shelf life by about 10%. On the other hand, Moreira, Ponce, del Valle, and Roura (2006) reported that mild heat shocks at 50 °C reduced the enzymatic browning of Romaine lettuce. Mild heat shock treatments is a new method developed to control browning reactions. The ease with which a heat shock can be administered to lettuce and the lack of an offensive chemical residue make this technique an attractive alternative to preserve fresh cut lettuce (Saltveit, 2000). Loaiza-Velarde et al. (1997) reported that dipping lettuce in water at 45–55 °C extends the shelf life and visual quality of minimally processed lettuce by inhibiting the activity of PAL. Taking into account that fresh horticultural crops differ in morphological structure, in composition and in general physiology, it is clear that requirements and recommendations for maximum shelf life will differ (Roura et al., 2003). In addition, the effectiveness of chemical dip treatments to increase postharvest storage life is affected by pH, temperature, water activity, microbial load and storage temperature. When different additives are used together, they may cause synergic, additive or antagonistic effects (Wiley, 1997).

The purpose of the present work was to determine the evolution of PAL activity in fresh cut Romaine lettuce after the combined application of mild heat shocks and chemical additives: calcium (1 g/100 g) or ascorbic acid (1 g/100 g). Heat shocks in chlorinated water combined with calcium chloride and ascorbic acid were applied before and after lettuce cutting operation.

2. Materials and methods

2.1. Sample preparation

Romaine lettuce (*Lactuca sativa* var Col) was obtained from a local producer and was taken to the laboratory where it was processed immediately. Lettuce heads were harvested at optimal maturity when they had reached a marketable size (approximately 20–24 leaves per head). Wrapper and core leaves were discarded. The remaining leaves were sorted for homogeneity in size and color and lack of defects.

The sequence of operations performed on lots A and B are presented in Table 1 both lots only differ when heat