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Novel insights into ascorbate retention and degradation during the washing and post-harvest storage of spinach and other salad leaves

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ABSTRACT

Post-harvest treatments of pre-packaged salad leaves potentially cause L-ascorbate loss, but the mechanisms of ascorbate degradation remain incompletely understood, especially *in planta*. We explored the extent and pathways of ascorbate loss in variously washed and stored salad leaves. Ascorbate was assayed by 2,6-dichlorophenolindophenol titration, and pathways were monitored by ^{14}C-radiolabelling followed by high-voltage electrophoresis. All leaves tested showed ascorbate loss during storage: lettuce showed the greatest percentage loss, wild rocket the least. Spinach leaves were particularly prone to losing ascorbate during washing, especially with simultaneous mechanical agitation; however, washing in the presence of hypochlorite did not significantly increase ascorbate loss. In spinach, [^{14}C]oxalate was the major product of [^{14}C]ascorbate degradation, suggesting that commercial washing causes oxidative stress. This study highlights that ascorbate/dehydroascorbic acid are lost via the oxidative pathway during washing and post-harvest storage of salad leaves. Thus changes to washing procedures could potentially increase the post-harvest retention of ascorbate.

1. Introduction

Vitamin C, comprising L-ascorbate and dehydro-L-ascorbic acid (DHA), is chemically the simplest vitamin. Unlike humans, plants can synthesise ascorbate, accumulating it at up to millimolar concentrations such that it accounts for up to 10% of the total water-soluble 'carbohydrates' (Noctor & Foyer, 1998).

Vitamin C participates in collagen synthesis (Mandl, Szarka, & Bánhegyi, 2009). Other reported health benefits include the treatment or prevention of diabetes, cardiovascular disorders, age-related diseases and cancer (Ames, Shigenaga, & Hagen, 1993; Mandl et al., 2009). Ascorbate is an antioxidant, but also has numerous other roles in plants including as an enzyme co-factor (Gallie, 2013), and in regulating the cell cycle (Smirnoff, Wheeler, & Loewus, 2000). In plants, apoplastic ascorbate may also play a beneficial pro-oxidant role, generating reactive oxygen species e.g. hydroxyl radicals (Fry, 1998), which may serve to loosen the cell wall during fruit ripening (Airianah, Vreeburg, & Fry, 2016).

Up to 90% of our dietary vitamin C is plant-derived (Lee & Kader, 2000) but cooking generally destroys much of the ascorbate in food (Lee & Kader, 2000). Therefore raw salads are an invaluable source of ascorbate. The ascorbate content of salad plants varies hugely, e.g. from 110 mg (curly kale) to as little as 3 mg (wholehead iceberg head lettuce) per 100 g fresh weight (McCance & Widdowson, 1991). Ascorbate content can also vary between cultivars of the same species (Hodges & Forney, 2003; Koh, Charoenprasert, & Mitchell, 2012; Ren et al., 2013), and younger plant tissues often have higher ascorbate concentrations than older ones, e.g. in spinach (Bergquist, Gertsson, & Olsson, 2006) and celery (Huang et al., 2016), presumably related to the ascorbate's role in plant growth.

The washing process of pre-packaged salads is also a potential source of ascorbate loss. Most commercial washing processes use recirculated water, treated with a sanitiser (e.g. chlorine-based). Iceberg lettuce washed in chlorinated water showed a marked decrease in ascorbate content after just one day's storage compared with lettuce washed in non-chlorinated water

Abbreviations: DCPIP, 2,6-dichlorophenolindophenol; DHA, L-dehydroascorbic acid; DKG, diketo-L-gulonate; HVPE, high-voltage paper electrophoresis; OxT, oxalyl-L-threonate.

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(Kenny & O'Beirne, 2009). However, spinach leaves washed in chlorinated water did not show any rapid ascorbate loss (Karaca & Velioglu, 2014). Conversely, washing with chlorine-based sanitisers caused considerably more ascorbate loss during subsequent storage than washing in peroxyacetic acid-based sanitisers, although a water-only control was not included (Gómez-López, Marín, Medina-Martínez, Gil, & Allende, 2013). Equally, spinach washed in chlorinated water showed greater loss of antioxidant activity than when washed in oxalic acid (Cefola & Pace, 2015), probably owing to the oxidising nature of chlorine-based sanitisers. These somewhat contradictory results suggest a need for further investigation of chlorine effects in the spinach.

Other steps in the processing of pre-packaged salads could also lead to ascorbate loss. For example, the slicing sometimes used on iceberg lettuce influences ascorbate content throughout shelf life, with hand-torn leaves showing higher ascorbate retention than blade-cut leaves (Barry-Ryan & O'Beirne, 1999), presumably because blades cause more severe wounding, leading to ascorbate consumption during the wound response.

Although vitamin C has been widely studied for many decades, much remains unclear about its degradation pathways. The first relatively stable degradation product of ascorbate is DHA. The oxidation reactions involved are effectively reversible in plants owing to the presence of DHA reductase and monodehydroascorbate reductase (Foyer & Halliwell, 1977; Truffault, Fry, Stevens, & Gautier, 2017). DHA can then be further oxidised to a range of products (Fig. 1), or hydrolysed to form diketogulonate (DKG), both these reactions representing a permanent loss of vitamin C from the plant tissue. DKG can itself be reduced to a redox-reactive substance with the formula $C_6H_6O_5$ (Kärkönen, Dewhirst, Mackay, & Fry, 2017), and DHA can be further oxidised, e.g. to oxalyl threonate (OxT) and cyclic oxalyl threonate (Fig. 1) (Green & Fry, 2005; Parsons, Yasmin, & Fry, 2011). Some plants accumulate ascorbate oxidation products, e.g. L-threarate (L-tartrate) in grapes

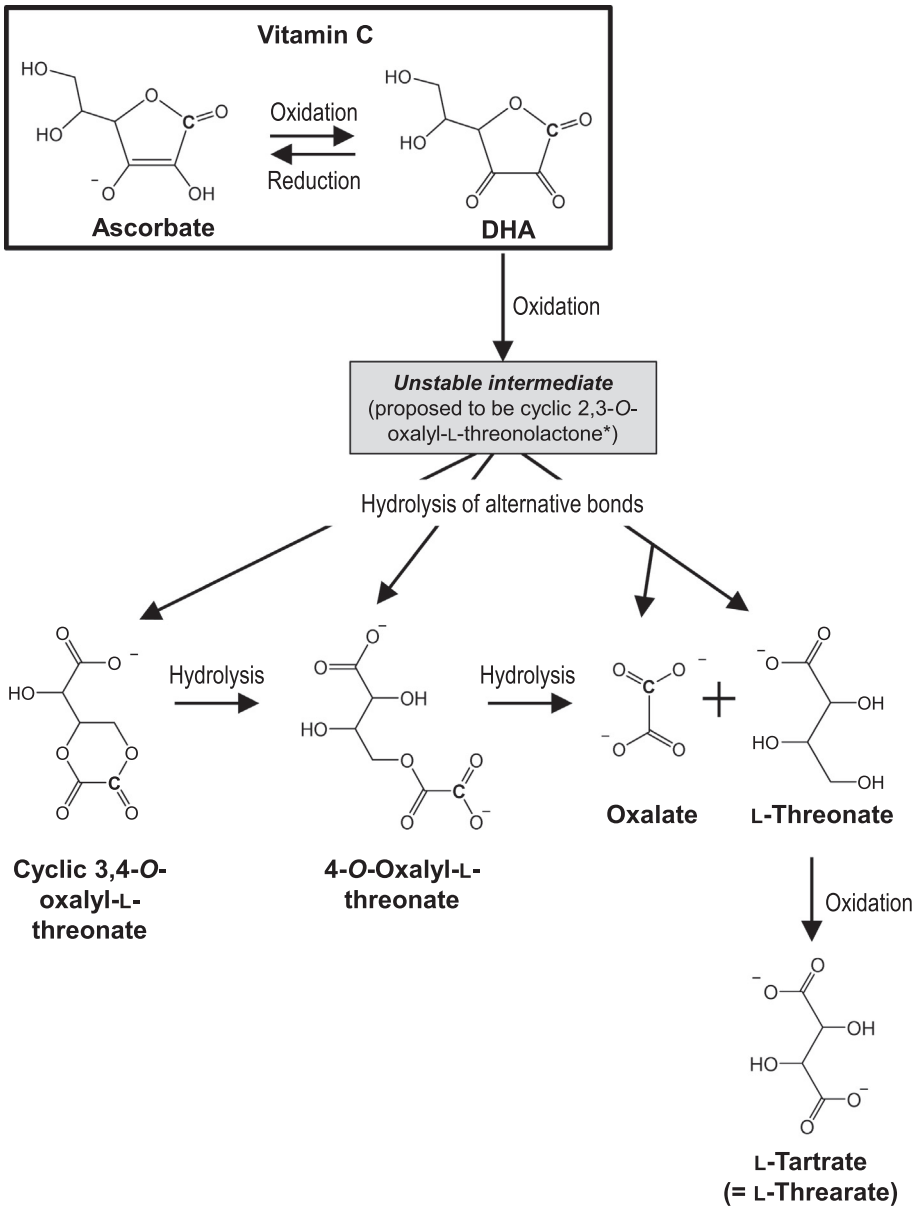

Fig. 1. The oxidation pathway of ascorbate. Vitamin C consists of ascorbate (AA) and dehydroascorbic acid (DHA). Further degradation of DHA, e.g. by the oxidation reactions shown here, results in a loss of vitamin C activity. The initial oxidation step between AA and DHA is effectively reversible in plants owing to the presence of DHA reductase. The C shown in the structural formulae indicates the radiolabelled carbon derived from the [1-^{14}C]AA used in this study. Pathway simplified from Parsons, Yasmin, and Fry (2011).