Identifying traits to improve postharvest processability in baby leaf salad

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Abstract

The ‘processability’ of baby salad leaves may be defined as the ability to withstand the postharvest washing and packing processes that are involved in the production of ready-to-eat bagged salads. The inability of baby salad leaves (species including \textit{Lactuca sativa} L. and \textit{Spinacia oleracea} L.) to withstand processing results in a reduction in crop shelf-life. Leaves from geographically diverse locations displayed strikingly different processability scores from visual inspection. We have shown that these ‘good’ and ‘poor’ quality leaves may be differentiated from assessments of the biophysical properties of the cell wall (% plasticity) and epidermal cell size. Artificial manipulation of processability in the glasshouse through the application of a mechanical stress or a high salt stress produced \textit{L. sativa} cv. Ravita (a leaf type ‘lollo rosso’ lettuce) leaves covering a range of processability. Mechanical stress, applied as a daily dose of 100 paper strokes, increased lettuce leaf shelf-life by 33% and was associated with reduced % plasticity and smaller leaf epidermal cells. These traits are thus proposed to be of key importance in the description of processability, with the plant cell wall and plant cell wall gene expression implicated. The potential for future manipulation of these traits for the pre-packed salad market is considered.

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Keywords: Baby salad; Cell wall extensibility; Cell size; Leaf physiology; Processability; Shelf-life

1. Introduction

The pre-packed baby leaf salad market was worth more than $1.6 billion worldwide in 1999, an increase of 15.9\% on the previous year (International Fresh-Produce Association), and it seems likely that this trend will continue for sometime. As the demand for this convenience food increases so too does consumer expectation and the need for a product in perfect condition, blemish free and fresher for longer. As farming methods, handling chain, washing equipment and packing become highly optimised to handle the leaves without causing excessive damage, further improvement must focus on leaf properties and postharvest
quality. It is highly likely that leaves with improved quality will be more able to withstand the rigorous processing that includes harvest, transportation, washing, sanitisation, de-watering and packaging. Such leaves should thus display an improved processability. Processability is the key factor in the commercial success of the industry.

Processing that causes damage often leads to browning of the leaves through breakdown due to a series of changes in phenolic metabolism. In iceberg lettuce varieties increase in phenylalanine ammonia-lyase (PAL) enzyme activity leads to an increase in the soluble phenolic content of the leaf that is turned brown by polyphenol oxidase (Ke and Saltveit, 1989). Methods to reduce this browning are under investigation, such as exposure to heat shock (Loaiza-Velarde and Saltveit, 2001), or a controlled atmosphere within the baby salad pack (López-Gálvez et al., 1996). In addition to discolouration, the physical characteristics of texture and firmness are also important in the processability of a baby salad leaf.

The textural properties of most foods from plant sources depend on the structure and integrity of the cell wall (Jackman and Stanley, 1995). Environmental stress can have dramatic effects on leaf physiology, growth and development, and it is possible that manipulation of this response could be utilised to enhance processability. Short term salt stress inhibits leaf growth and expansion in maize without significantly affecting the physical properties of the cell wall (Cramer et al., 2001), although other reports (Neumann et al., 1988), suggest that the wall undergoes mechanical adjustment leading to increased extensibility. Moderately salt stressed lettuce leaves (10 mM sodium chloride) displayed altered patterns of nutrient transfer, with an increase in sodium and chloride ions transferred to the younger leaves and a reduced calcium ion transfer, leading to an inhibition in growth (Lazof and Bernstein, 1999). Mechanical stress reduces leaf elongation and improves tolerance to other stresses (Pöntinen and Voipio, 1992). Mechanical stress (‘touch’) has been used to control the growth of lettuce and cauliflower seedlings (Pöntinen and Voipio, 1992), and lettuce, cauliflower and celery seedlings (Biddington and Dearman, 1985). In these experiments daily brushing resulted in modified leaf architecture, producing smaller, more compact leaves that may show greater processability, but this has never been tested. Touch has been shown in Arabidopsis thaliana L. Heyn to induce a group of cell wall modifying enzymes, xyloglucan endotransglycosylases (XETs) (Fry et al., 1992), responsible, amongst other functions, for strengthening the cell wall (Campbell and Braam, 1999).

The aim of the research reported here was to characterise leaf traits associated with improved postharvest processability in baby salad leaves, with the long-term goal of breeding baby salad crops with the desired traits. This paper presents: (i) a series of measurements made on ‘good’ and ‘poor’ material across a range of contrasting species selected from different geographical locations; (ii) two experiments where processability was manipulated in Lactuca sativa L. ‘lollo rosso’ through the burden of stress, either by salinity or a mechanical treatment.

2. Materials and methods

2.1. Plant material

Commercially produced ‘baby leaves’ of Lactuca sativa L. (lettuce) types ‘lollo rosso’ and ‘cos’ and Spinacia oleracea L. (spinach) were used in the study. Leaf samples were from a range of farms: high quality lettuce from Timau, Kenya (‘Good’); spinach and poorer quality lettuce from Odemira, Portugal (‘Poor’); high quality organic spinach from Salinas, USA (‘Good’) and lesser quality organic spinach from Verona, Italy (‘Poor’). Samples were assessed for quality by staff at a UK salad processing factory (Vitacress Salads Ltd., St. Mary Bourne, UK) where processing was performed using industrial washing, drying and packaging equipment.

For the stress experiment, L. sativa cultivar Ravita (a lollo rosso type lettuce) was grown under glass and subjected to stress treatments. Seeds of Ravita (Enza Zaden B.V., Enkhuizen, Holland) were germinated in 125 cm³ cells using a compost mixture (medium grade Vermiperl Vermiculite:
Levingtons F2, 2:1; v/v) (Avon Crop, Bracknell, UK) with conditions set for 20 °C by day and 14 °C by night, with a supplementary lighting source to give 16 h days, good air circulation and regular watering.

2.2. Stress treatments

For the salt stress experiment seedlings were thinned out to one per cell after germination and following the appearance of true leaves the trays were watered from below with either 50 mM sodium chloride (Fisher Chemicals, Fisher Scientific UK Ltd., Loughborough, UK), or tap water. All plants were watered once from above, with tap water, prior to harvest, 5 weeks after the seed was sown. The first two emerging leaves were discarded and the remaining leaves were labelled L1–L4, with L1 being the older outermost and L4 the younger innermost leaf.

For the mechanical stress experiment seedlings were thinned out to three per cell, selected for uniformity and watered at the base. Trays were treated with mechanical stress by stroking back and forth, 0, 10, 25, 50 or 100 times, with an A4 piece of paper (80 gm⁻²) folded to double thickness. Plants were treated between 12:00 and 14:00 daily for 15/20 days. Material was harvested from the centre of the trays to prevent edge effects and labelled as before.

2.3. Shelf-life and organoleptic assessment

Leaves were ‘processed’ in 20 l distilled water in a Hotpoint Supermatic Plus 9404 twin tub washing machine (General Domestic Appliances Ltd., Peterborough, UK) for 1 min on the lowest setting and dried for 20 s in the spin compartment. Fifty grams of the product were kept in zip-sealed polythene bags, and stored at 6–8 °C. Shelf-life was determined by a daily examination of the bags at 14:00, with rejection after three independent distinguishable signs of breakdown.

Organoleptic assessment was made on unprocessed L1 and L2 leaves bagged in pre-formed commercial salad film (Amcor Flexibles Europe, Ledbury, UK), heat-sealed and held at 4 °C. Leaves were assessed by panel at 0, 5 and 7 days post packing in terms of wetness, breakdown and bruising, discoloration and texture. Time points were chosen as key points in the commercial life of packaged salad products.

2.4. Leaf area and weight analysis

Leaf area was calculated by scanning the visible surface of the leaf on a flat bed scanner (Hewlett Packard 6100C) along with a known size marker. Scion Image software (Scion Corp., Frederick, USA) was used to determine the leaf area in mm².

Leaf fresh weight was recorded on a top pan balance and dry weight was measured after 48 h at 80 °C. Percentage dry weight was determined as ((dry weight)/(fresh weight)) × 100.

2.5. Biophysical testing and specific leaf area

Baby leaves were harvested immediately into 20 ml of methanol and stored in the dark at 4 °C until testing. A home-made Instron machine was used as described by Ferris et al. (2001), with a number of modifications. A 20 g weight was used for the load, and re-hydration was performed in an 256 cm³ vessel, containing 150 ml of distilled water, on a shaker (Mk V Orbital Shaker, LH Engineering Co. Ltd., Stoke Poges, UK) at a mid setting for 10 min. The leaf section was cut parallel to the mid-vein 10 mm across from the vein and down from the leaf tip. The 5 mm wide and 10 mm long strip was blotted dry and stretched vertically between clamps set 5 mm apart. Percent elasticity (reversible extension) and % plasticity (irreversible extension) were determined from the chart output for the load. Stretched sections were oven-dried at 80 °C to calculate specific leaf area (SLA) of section, SLA = (leaf area)/(dry leaf weight).

2.6. Epidermal cell area measurement

A leaf disc was taken parallel to the mid-vein 10 mm in from the vein and tip. The disc’s adaxial surface was blotted dry and coated in clear nail varnish (‘3 in 1’ Nail Care, Rimmel, London, UK). The dried imprint was peeled from the disc using ‘sellotape’ and transferred, along with the tape, to a glass slide (protocol modified from Gardner et
al., 1995). The image was captured using a video camera attached to a light microscope and exported to Scion Image (Scion Corp., Frederick, USA) for analysis. All complete epidermal cells were traced, with the exception of those bordering stomatal complexes, and used for mean cell area calculation per captured image.

2.7. Cell sap osmolarity

Osmolarity measurements were made on a Wescor 5100C vapor pressure osmometer (Wescor Inc., Logan, USA) from leaf sap samples. Sap was collected from 5 g of fresh leaves using a kitchen garlic press. Samples were spun at maximum for 15 s in a small bench centrifuge and the supernatant was then immediately tested following the manufacturer’s instructions.

2.8. Statistical analysis

One way analyses of variance was used throughout. Levels of significance, were shown as $F$ values and depicted as $P < 0.05$, *; $P < 0.01$, **; $P < 0.001$, ***.

3. Results

3.1. ‘Good’ versus ‘poor’ material

Harvested baby leaf material, from a range of geographic locations, classified as having ‘good’ postharvest processability were compared with the same crop classified as ‘poor’ quality in postharvest processability (Table 1). Here, three leaf characteristics were investigated in detail: (i) epidermal cell area; (ii) cell wall extensibility (% elasticity and % plasticity); (iii) osmolarity of cell sap. All three characteristics were considered important for postharvest processability from a preliminary study (data not shown). Across the crops studied, the only significant difference between leaf samples of different quality for epidermal cell area was with the leaf lettuce type lollo rosso. Epidermal cell imprints were larger (2014

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<th>Table 1</th>
<th>Comparisons between material graded ‘good’ or ‘poor’ for leaf traits associated with postharvest processability</th>
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<td>Factor</td>
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<td>Epidermal cell area</td>
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In each experiment ‘good’ and ‘poor’ material was graded and collected from the processing facility. Units: Epidermal cell areas ($\mu m^2$); % plasticity (wall strain per 20 g force exerted); osmolarity ($mmol kg^{-1}$). Values represent means + standard error, results of one-way ANOVAs are shown. $G$ = ‘good’ quality processability, $P$ = ‘poor’ quality processability.
\( \mu m^2 \) in the good quality leaves than the poor quality leaves (1476 \( \mu m^2 \)). There was no significant change in cell size between samples of good and poor quality in organic spinach and the leaf lettuce type cos.

Cell wall properties for ‘good’ and ‘poor’ differed. Highly significant changes in plasticity (\%P), were particularly apparent. ‘Good’ quality lollo rosso material had an irreversible extension (\%P) of 2.56\% compared to the poor material’s 4.58\%. The pattern was also observed in organic spinach, where the plasticity of ‘good’ material was lower (3.35\%) than that of the leaves that exhibited ‘poor’ processability (6.65\%). Reversible extension (elasticity) did not change significantly between material graded differently in terms of processability. In summary ‘good’ quality baby leaves all had less plastic cell walls.

The osmolarity of cell sap extracted from leaf samples of ‘good’ and ‘poor’ quality differed significantly. The osmolarity of lollo rosso leaves from ‘good’ material was 103 mmol kg\(^{-1}\) which was significantly higher than 72 mmol kg\(^{-1}\) for cell sap from ‘poor’ quality leaves. A similar observation was made for organic spinach, where the ‘poor’ quality leaves had an osmolarity (223 mmol kg\(^{-1}\)) that was significantly lower than the ‘good’ quality leaves (275 mmol kg\(^{-1}\)). However, in contrast with cos, the osmolarity was significantly different but an increased osmolarity was found in the ‘poor’ quality material.

In the second part of the study on harvested baby leaf material comparisons were made between unprocessed leaves and leaves post processing (Table 2). Two samples of lollo rosso were assayed, one was graded professionally as ‘excellent’ in terms of processability (sourced from Kenya) and the second sample graded ‘poor’ (sourced from Portugal). The ‘excellent’ material did not change following processing; cell sap osmolarity (unprocessed 103 mmol kg\(^{-1}\), post processing 104 mmol kg\(^{-1}\)) and cell wall material properties (unprocessed \%P 1.55\%, post processing \%P 1.57\%) remained constant. The ‘poor’ quality material was changed by the process, \%P decreased (not significantly) and solute concentration also increased (highly significant). These changes indicate that the process caused changes to the cell wall material properties and altered the cellular solute levels in the ‘poor’ quality samples. These changes are likely to be caused by mechanical damage and water damage during the process and affect the product post processing.

Processing caused changes in the properties of cos and spinach baby leaves. Elasticity and plasticity percentages increased post processing in cos with highly significant movement for elasticity (from 3.45 to 4.60\%) and very significant changes in irreversible extension (\%P from 1.20 to 2.44). In spinach plasticity decreased following processing from 4.82 to 4.12\%, in contrast to the leaves of lollo rosso and cos where the irreversible extension was increased by the process. Solute concentration in cos and spinach decreased significantly after the process, in comparison to lollo rosso leaves. However, in all three baby leaves the process caused changes in all but the most processable baby leaves.

3.2. Using salt stress to manipulate postharvest processability

Plants were watered with either tap water (control) or 50 mM sodium chloride, in order to assess effects on postharvest processability traits. Leaf biophysical properties were examined (Fig. 1) using an Instron device. The reversible extension (% elasticity) did not change following the stress application but the irreversible extension (% plasticity) was increased with the salinity treatment from 1.52 to 2.52\% and this was statistically significant. Cell wall properties were therefore altered by the application of salt stress, resulting in a higher value of \%P.

Cell sap osmolarity in the salt stressed plants (Fig. 2A) was highly significantly increased to 942 mmol kg\(^{-1}\) (from 281 mmol kg\(^{-1}\)). The sap from stressed leaves contained nearly four times the solutes of the control leaves. Solutes were retained in the stressed leaves, where there was less water. Three further measurements of leaf traits associated with postharvest processability were made on the outer harvested leaf. Epidermal cell area (Fig. 2B) was reduced by 45\% to 1555 \( \mu m^2 \) in the stressed plants. SLA, a measure of the thickness of the leaves, was reduced by 40\% in the salt stressed
leaves (Fig. 2C), indicating an increase in thickness, where less surface area has an equivalent weight. The leaf area was highly reduced in the stressed leaves (Fig. 2D); the fresh weight per leaf was halved, but the dry weight as a percentage of the fresh weight was doubled in the stressed leaves (data not shown).

The stressed leaves were smaller, but thicker, with an increased dry weight ratio. The impact of these changes on the postharvest quality of the product was made by an organoleptic assessment of packaged leaves, (Fig. 3). The stressed leaves scored considerably higher on the organoleptic scale than leaves from plants given the control treatment. At the point of harvest the stressed leaves were given a higher mark than the control treatment (99.2 and 93.5%, respectively) and 7 days postharvest the salt stressed leaves scored 50% higher than the treatment. The product had a longer shelf-life in terms of consumer acceptability following growth under a salt stress regime.

3.3. Using mechanical (touch) stress to manipulate postharvest processability

Lollo rosso plants were treated with a second stress during glasshouse growth, again to manipulate the postharvest processability and related leaf physiological and biophysical properties. Plants were touch-stressed by stroking back and forth with a folded piece of A4 paper and leaf traits postharvest were measured. The first leaf area was significantly reduced following stress with 100 strokes per day from 1816 to 741 mm² (Fig. 4A) and the epidermal cell area in the stressed L1 leaves was also significantly reduced from 1482 to 1379 µm² (Fig. 4B). Leaf dry weight was increased following the stress, indicating a shift in resources to the leaf as a direct result of the stress (Fig. 4C).

Processability in terms of shelf-life post processing was measured in harvested lollo rosso leaves that had been subjected to a series of strokes per

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<th>Leaf traits associated with postharvest processability and the impact of the process on the traits</th>
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In each experiment material was collected from the processing facility before washing and packing and after washing and packing. Units: % plasticity (wall strain per 20 g force exerted); osmolarity (mmol kg⁻¹). Values represent means ± standard error, results of one-way ANOVAs are shown. Un = unprocessed material, Pr = processed material (washed, dried and packed).
day from 0 to 100. The leaves were washed, dried and bagged to replicate the industrial processing and stored in a refrigerator, where they were examined daily for signs of breakdown, leading to rejection. As the number of strokes per day increased the shelf-life was linearly increased (Fig. 4D). With the maximum number of strokes, 100, the shelf-life was 3 days longer than that of the control leaves, an increase of 50% in lollo rosso.

Shelf-life post processing was also increased by mechanical touch in baby leaves of spinach and salad rocket (data not shown). Over the range of treatments from 0 to 100 strokes epidermal cell area decreased linearly in relation to the extent of the stress (Fig. 5A). As the mechanical stress increased the epidermal cells and the leaves themselves were reduced in size. These changes had a dramatic effect on the processability of harvested leaves.

The effects of touch stress on the biophysical characteristics of leaves (Fig. 5B) was non-linear. With a small stress of 10 strokes the elasticity increased (to 16.1%), before decreasing below the control’s value (14.3%) as the stress was heightened (to 12.5% at 100 strokes). The plasticity of the leaves followed a similar pattern to rise from the control (7.40%) in the mid-stress treatments (10, 25 and 50 strokes) before dropping to 6.88% with 100 strokes.

4. Discussion

The most important finding of this research was the discovery that shelf-life may be increased in baby leaf lollo rosso by 3 days, from rejection at 6 days in the control to 9 days following a daily exposure to a brushing ‘touch’ treatment. We have shown that this was linked to a reduction in epidermal cell area and modifications to cell wall biophysical properties, measured as plasticity and elasticity. This is of great commercial interest and suggests that manipulation of these two characteristics—leaf size and cell wall properties—could significantly improve the shelf-life of a number of baby salad leaves.

Postharvest shelf-life was linearly linked to the dose of mechanical stress applied during plant growth. As the applied daily ‘touch’ increased so did leaf plasticity, up to a maximum of 50 strokes, beyond which the plasticity was reduced, but shelf-life was further extended. It is proposed that the application of ‘touch’ resulted in an up-regulation of genes to strengthen the cell wall and protect the leaf against the stress. The cell wall of the model plant Arabidopsis is strengthened by an increased incorporation into the cell wall of xyloglucan, the most abundant cell wall hemi-cellulose through activation of specific XET genes in response to touch (Braam et al., 1997). XET is also important for the loosening and expansion of the cell wall, but in this instance, different XET enzymes are thought to be relevant since 46 XET and XET-related (XTR) genes have been defined in a recent review by Campbell and Braam (1999). Mechanical stress gave rise to more compact lettuces, comprising smaller leaves (Fig. 4A), and smaller cells (Fig. 4B). As with cell wall plasticity, there was a strong correlation between cell area and the number of strokes applied in the touch treatment (Fig. 5A). Postharvest shelf-life of the leaves was related to the epidermal cell size, with smaller cells exhibiting an extended life, although this effect
appeared to reach a ‘maximum’ at 50 strokes per day. Wurr et al., (1986), showed that brushing of lettuces resulted in leaves of reduced length and weight, enhancing handling traits in the transplanting of plants from glasshouse to field. Our data on baby salad leaves confirm these findings, but make a significant new addition in demonstrating the positive effect of touch on baby leaf processability.

Our data support the suggestion that a leaf with many small cells may be more processable than a larger leaf with the same number of cells, due to the increase in cell wall volume of the leaf containing many small cells, giving the leaf tissue a higher density, as we have shown with an increased dry weight, and improved firmness. The epidermal cells on the surface of the leaf are a good indicator of cell sizes in the leaf with the epidermis controlling the growth of the whole leaf (Kutschera, 1992). The ‘touch’ stress did not result in any significant changes to the thickness of lollo rosso leaves (data not shown), in contrast to Biddington...
and Dearman (1985), where the ratio of weight to surface area in their touch-stressed lettuce leaves was altered, changing the thickness of the leaves to maintain turgor pressure.

Salt stress also resulted in an improved baby leaf processability. The leaves harvested were smaller and thicker, consisting of smaller cells (Fig. 2C,D), and displayed an increased plasticity (Fig. 1). This is in contrast to the decreased plasticity observed in the touch experiment, but has been observed previously in leaves exposed to salt stress. Despite this difference in response, these changes in the lollo rosso leaves gave rise directly to an extended shelf-life (Fig. 3), and thus the reported improved processability. Salinity reduces leaf elongation in maize (Munns et al., 2000), and leaf cell expansion (Cramer et al., 2001), without affecting extensibility. However, in agreement with our results of an increased %P, Neumann et al. (1988) exposed beans to a 10 day salinity stress prompting an increase in cell wall extensibility. Lettuce leaf growth has been previously reduced by salt stress (Lazof and Bernstein, 1999), and salt stress reduces yield in lettuce (Tarakcioglu and Inal, 2002). Changes in leaf size and yield are not relevant to the production of baby leaf salad and the research finding reported here could be commercially applied to keep leaves within size specifications for longer, with the reduced yield negated by the increased quality of the product and a reduction in discarded, unprocessable, crop.

In assessing the general relevance of our findings on touch and salt stress, we also investigated the hypothesis that leaf cell size and cell wall properties differed between ‘good’ and ‘poor’ field grown material. In general, cell wall plasticity was lower in ‘good’ material, confirming our finding from the stress experiments, whilst solute content was higher in two out of three species studied. Epidermal cell size differed less in these analyses of ‘good’ and ‘poor’ material. A further comparison between processed and unprocessed material from the same harvest was also undertaken to determine the impact of the process on traits identified to be important for postharvest processability and to assess whether ‘good’ material was able to withstand the processing treatments better than ‘poor’ material. The most interesting finding was that for ‘excellent’ and ‘good’ material, leaves appeared more able to withstand the process of washing...
than the ‘poor’ material. The process caused changes in the cell wall properties of all the leaves, changing %P and processing damage was also shown to lead to the leakage of cellular fluids in red chard (Roura et al., 2000).

The cell wall consists of two elements—the elastic and inelastic plastic properties. The elastic parts of the cell wall return to their original shape after tension but the plastic elements are sheared irreversibly by the load strain applied. For processability the stress experiments increased the plasticity of the leaves such that they were able to withstand a greater force before they sheared. It can therefore be assumed that the strengthened cell walls confer an increased stiffness, (Cosgrove, 1993), and this results in a more processable leaf.
that suffers less processing damage. Processed leaves have a higher %P than the unprocessed baby leaves. There is a degree of conflict between the analysis of the plasticity, with the ‘good’ material having a significantly reduced plasticity and the most extreme mechanical stress also having a reduced plasticity. It is proposed then that other, as yet unidentified, traits also contribute significantly to baby leaf processability, in addition to leaf plasticity and these remain to be elucidated. Despite this, the research reported here suggests that there is considerable potential to target the cell wall for further breeding and improvement in baby salad leaves and this could be achieved through both traditional breeding and biotechnological approaches.

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References


