Horticulture Innovation Australia

Final Report

Development of Shelf life indicators for baby leaf spinach and rocket

Gordon Rodgers
AHR Environmental Pty Ltd

Project Number: VG08166

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Development of Shelf Life Indicators for Babyleaf Spinach and Rocket

CONFIDENTIAL

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Horticulture Australia
Project Number: VG08166

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The purpose of this project was to further our understanding of shelf life in babyleaf spinach and to assess how well a range of indicators could be used to predict shelf life with a view to developing screening tests for plant breeders and field tests for growers.

This project has been funded by HAL using voluntary contributions from industry and matched funds from the Australian Government.

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1 Media Summary

Shelf life is an issue of critical importance in babyleaf spinach because it relates directly to freshness, a quality that consumers value highly in their buying decisions.

Breeders are expected to produce varieties with good shelf life characteristics. And growers are expected to supply crops to the market and to processors with a long shelf life, to reach consumers in good condition. However, no techniques have been available to help breeders or growers to reliably assess shelf life characteristics of new babyleaf spinach varieties or of crops in production.

The project identified the relative importance of damage during harvesting and processing on shelf life, the reasons for babyleaf spinach failing shelf life, and the length of time spinach could be successfully stored under ideal growing and storage conditions for summer- and winter-grown crops. The project also identified and evaluated a range of tests that could be used to predict shelf life by plant breeders or growers. Useful tests included specific leaf area, leaf colour, leaf respiration rate, chlorophyll, leaf thickness, plant height, total leaf area, amount of leaf damage and leaf apex tearing.

The most useful indicators of shelf life should be fine-tuned and adopted as predictive tests both for in-field use, and as breeding selection tools for shelf life. More work should be done on determining the effect of high nitrogen availability and planting density on the shelf life of spinach under different seasonal conditions.

Two significant areas of research remain to be completed. First, the development of reliable tests that growers can use to predict what the shelf life of a crop that is in the ground will be once it is harvested, when it has been affected by adverse conditions during growth. Second, actions that can be applied to adversely affected babyleaf crops before harvest to restore the shelf life to an acceptable level should be evaluated under conditions known to induce poor shelf life, i.e. summer in southern Australia.

2 Technical Summary

Shelf life is an issue of critical importance in babyleaf spinach because it relates directly to freshness, a quality that consumers value highly in their buying decisions.

Breeders are expected to produce varieties with good shelf life characteristics. And growers are expected to supply crops to the market and to processors with a long shelf life, to reach consumers in good condition. However, no techniques have been available to help breeders or growers to reliably assess shelf life characteristics of new babyleaf spinach varieties or of crops in production.

AHR worked in collaboration with vegetable seed company Rijk Zwaan to develop these objective measures, and attempted to gain a better understanding of how factors such as variety and growing environment affect shelf life.

The main focus of the project was on spinach because this is the major babyleaf species grown in Australia. Spinach varieties were selected that have a wide range in shelf life, i.e. short- to long-lasting after harvest. These varieties were then grown under cool or warm conditions, to impose the influence of growth rate or season on shelf life.

The spinach crops were mechanically harvested, and then processed through a commercial washing and packing facility. The levels of damage of the harvested leaf were assessed before and after processing. The leaves were stored at 5^{o}C in low-density polyethylene bags and shelf life was assessed daily after initial storage for 7 days. A wide range of potential predictors of shelf life was measured as part of the experiment. These included specific leaf area, individual and total leaf area, leaf thickness, elasticity, structural and non-structural carbohydrates including individual sugars, chlorophyll, leaf colour, respiration rate, stomatal density, and leaf epidermal cell area.

For rocket, the work focussed on measuring the impact on shelf life of rocket type (arugula or European wild rocket), nitrogen supply in the field and storage temperature after harvest from crops grown in spring, summer or autumn at Camden, NSW. The visual quality of leaves grown during different seasonal and multi-harvest events was determined at different storage temperatures (0, 4, and 7°C). The shelf life of rocket species was also measured for leaves grown at nitrogen supply levels of 0, 50, 150, and 250 Kg N ha^{-1} and stored at 0°C.

The key findings for spinach were:

- Moderate bruising caused by harvesting and processing recovers after 7 days in storage at 5^{o}C but severe bruising does not recover and leads to a loss of shelf life.
- Damage caused by processing does not have a major effect on shelf life provided the equipment is of good quality. Excessive damage during processing will cause loss of shelf life as above.
- The loss of shelf life due to desiccation in the first two experiments is not likely to occur commercially because bags are usually sealed.
- Extremely long shelf life is possible, up to 50 days after harvesting when held at 5^{o}C, with minimal damage (either processed or unprocessed).
- Most leaf failures are due to microbial breakdown, not loss of chlorophyll.

- Useful indicators of potential shelf life in order of significance were:
    - Variety
    - Growth rate (season)
    - Specific leaf area (negative)
    - Leaf colour at harvest or after 7 days (positive)
    - Respiration rate at 20^{o}C or 5^{o}C (negative)
    - Total chlorophyll (b) on fresh-weight basis (negative)
    - Plant height (negative)
    - Leaf thickness (positive)
    - Leaf area (total and individual) - negative
    - Stomatal density (positive)
    - Total leaf damage (negative)
    - Leaf apex tearing and splitting (negative)

- Characteristics not predictive of shelf life:
    - Leaf elasticity
    - Epidermal cell size
    - Starch, sucrose, fructose or glucose levels
    - Total non-structural carbohydrate levels
    - Chlorophyll on leaf area basis
    - Minor leaf bruising

The key findings for rocket were:

The shelf life of both rocket species was significantly influenced by storage temperature, with storage at 0°C consistently resulting in a longer shelf life. The longest shelf life of European wild rocket was 26 days after harvest for summer-grown leaves stored at 0°C. In comparison, the longest shelf life of arugula was 22 days for winter-grown leaves stored at 0°C. When stored at 7°C the shelf life of European wild rocket was significantly longer for leaves grown during summer, which achieved a shelf life of 17 days. During winter, leaves had a shelf life of 16 days, and during spring 12 days. Under the same storage conditions arugula had a longer shelf life for leaves grown during winter, which had a shelf life of 15 days, followed by spring with a shelf life of 14 days, and summer 13 days.

When stored under similar conditions European wild rocket had a slightly longer shelf life than arugula, confirming perceptions within the industry. However, under commercial storage conditions of 7°C the shelf life of arugula (±2 days) was more consistent than European wild rocket (±5 days) across seasonal conditions, indicating that this species may be better suited to the year-round supply of leaves within the current commercial supply chain.

The most useful indicators of shelf life should be fine-tuned and adopted as predictive tests both for in-field use, and as breeding selection tools for shelf life. More work should be done on determining the effect of high nitrogen availability and planting density on the shelf life of spinach under different seasonal conditions.

Two significant areas of research remain to be completed. First, the development of reliable tests that growers can use to predict what the shelf life of a crop that is in the ground will be once it is harvested, when it has been affected by adverse conditions during growth. Second, actions that can be applied to adversely affected babyleaf crops before harvest to restore the shelf life to an acceptable level should be evaluated under conditions known to induce poor shelf life, i.e. summer in southern Australia.

3 Introduction

The babyleaf sector is a significant part of the leafy vegetable market in Australia. It was valued at $M25 in 2009 (ABS) and increasing rapidly, with a current value estimated at closer to $40M per annum. The industry is made up of independent growers, grower-processors, growers supplying processers, and processing companies.

The babyleaf sector comprises a large number of different leafy vegetable crops, which are all harvested as young plants and can be used as salad vegetables. Spinach dominates the industry with a much higher level of production compared to any of the other species.

Breeders are expected to produce varieties with good shelf life characteristics. And growers are expected to supply crops to the market and to processors with a long shelf life, to reach consumers in good condition.

The issue of shelf life was first investigated in Australia as part of HAL project VG05068. This project investigated the impact of factors such as variety type, growing region and season, planting density, postharvest handling, nitrogen nutrition and establishment method on yield and shelf life.

One of the key findings of project VG05068 was that growth rate had a major effect on the postharvest shelf life of harvested spinach. The best shelf life was achieved when spinach took more than 32 days to grow from sowing to harvest and the shelf life was reduced by about half a day for every day reduction in growth rate below 32 days.

The critical growth period of 32 days or more from sowing to harvest can be achieved in two ways. Crops can either be grown in a cool environment that will slow the growth rate, or by using slow-growing varieties in warm environments.

For warm environments such spring and summer in Victoria or NSW, the best shelf life can be achieved by using slow-growing commercial varieties such as Crocodile, Island or Sardinia. In cool environments such as winter in Victoria or NSW, faster-growing varieties can be used such as Parrot or Nighthawk to give an acceptable balance between quality and productivity. There are also varieties that are more suited to intermediate climate, or the so-called “transition” production time. Varieties with an intermediate growth rate include Roadrunner, Ibiza and Wallis.

There also appears to be a relationship between minimum average night temperature and shelf life in spinach. The closer the night temperature is to 7^{o}C, the better the shelf life, and night temperatures above 15^{o}C result in unacceptable shelf life.

These results support the work of Boese & Huner (1990) who found that cooler growing conditions improved postharvest spinach quality. During cooler nights the respiration rate of the leaves will be lower than if the night temperatures are warm and this means that there will be a greater accumulation of stored carbohydrate, which can be used for growth (Cantwell and Suslow, 2002; Ueda et al., 1998).

There were some attempts in project VG05068 to measure potential indicators of shelf life, and one of these that showed some promise was leaf thickness. There was a consistent positive correlation between leaf thickness and shelf life, and this was observed in response either to manipulating variety type and or to different seasonal conditions. Slower-growing varieties and cool climates resulted in thick leaves with good shelf life, and fast-growing types and warm weather resulted in thinner leaves with poor shelf life.

This finding led to the idea that leaf thickness, or possibly a whole range of other characteristics, might be useful predictors of shelf life either for growers or for plant breeders.

The other important idea was to look more deeply into the relationship between growth rate/variety and shelf life and see what could be concluded in relation to breeding for better spinach, or perhaps adopting growing techniques that would result in better quality product.

With regard to the other main babyleaf crop, rocket, either European Wild Rocket (*Diplotaxis* sp.) or cultivated or arugula rocket (*Erica sativa*), there has been scant research done on factors that might be expected to influence shelf life, such the influence of season, nitrogen supply or storage temperature. The opportunity therefore was present for the project to make a contribution in that area.

The project was developed to look more closely at factors that affect shelf life in baby spinach, especially at the plant physiology level, and then to test a range of physical or biochemical parameters as potential indicators of shelf life, and which could be used by plant breeders to breed better spinach, or by growers to produce better crops. There was also a component of the project that looked at the impact of nitrogen supply and postharvest storage temperature on the shelf life of the two types of rocket, aragula and European Wild Rocket.

4 Materials and Methods

4.1 Field location and experimental design

Four experiments were planted on a commercial babyleaf farm during 2010 and 2011 at Ellis Lane, approximately 70km southwest of Sydney, Australia (Table 1). The seeds of all varieties were planted at a density of 750 plants m^{-2}. Plants were grown on standard 1.5 m wide raised beds, with a plot length of 20m.

Table 1: Information on planting and harvest dates for respective experiments.

Experiment	**Number of varieties**	**Planting date**	**Harvest date**
1	25	12/03/2010	06/04/2010
2	25	24/08/2010	27/09/2010 & 05/10/2010
3	10	29/12/2010	21/01/2011
4	10	25/04/2011	24/05/2011

[a] GDD = growing degree days.

4.2 Growth characteristics

Varieties of babyleaf spinach were selected with a wide range in expected shelf life. This was the major source of shelf life variability on which the remaining studies were focussed (Table 2). Seeds were planted using a commercial babyleaf seeder (Seed Spider Seeding Systems, New Zealand). The weight, leaf area and leaf thickness of plants were measured at 14, 21 and 28 days after seeding (DAS); with 23 plants per variety sampled for these measurements. Whole plants were sampled with the roots cut off 2mm below the crown.

Once samples were taken back to the lab, individual plant weight was determined using an electric balance (Mettler Toledo PB303-S, Switzerland). Leaf area was then measured using a LI-3000C area-measuring meter attached to a transparent conveyer belt (John Morris Scientific, Australia). The thickness of medium-sized leaves was then measured using a micrometer (Mitutoyo, Japan). The average number of expanded leaves per plant was also recorded for individual varieties.

Table 2: Varieties of babyleaf spinach known to have a range in shelf life.

Experiment 1	Experiment 2	Experiment 3	Experiment 4
Zebu	Zebu	Toucan	Toucan
09.78387	09.78387	Donkey	Donkey
Pigeon	Pigeon	Whale	Whale
Toucan	Toucan	Emu	Emu
Donkey	Donkey	9.78201	9.78201
Marabu	Marabu	Crocodile	Crocodile
Silverwhale	Silverwhale	Squirrel	Squirrel
Swan	Swan	10.98151	10.98151
Whale	Whale	Turtle	Turtle
Emu	Emu	Racoon	Racoon
09.78201	09.78201		
Crocodile (semisavoy)	Crocodile (semisavoy)		
GB.25650	GB.25650		
Red kitten	Red kitten		
Squirrel	Squirrel		
9.78220	9.78220		
10.98084	10.98084		
10.98151	10.98151		
Turtle	Turtle		
Sparrow	Sparrow		
Roadrunner	Roadrunner		
Red cardinal	Red cardinal		
Pelican	Pelican		
Racoon	Racoon		
Parrot	Parrot		

4.3 Harvesting and processing

Once leaves reached commercial maturity they were harvested using a commercial babyleaf harvester (Grech engineering, Australia). The leaves were placed into 15kg plastic crates and cooled in a Thermfresh® cool room set at 2°C. Three replicates were used for pre-processing assessment. The remaining spinach was transported after cooling to the Golden State Foods (GSF) factory at Wetherill Park in Sydney where it was stored at 2°C until processing the following day.

The washing and drying step was conducted at the GSF processing facility using a commercial fresh-cut washer / drier (Photo 1). There were three washing steps and the leaf was dried using a commercial spin dryer before being lifted using an elevator and packed into non-MAP (modified atmosphere packaging) sealed polyethylene bags. Samples were packed using the commercial bagger and assessed using the same criteria as for the non-processed samples except that an additional damage assessment was made immediately after washing and drying.

4.4 Biophysical properties

These tests were assessed for their potential to predict differences in shelf life, induced either by variety or season or both.

4.4.1 Elasticity

The elasticity of mature leaves was examined in the field before harvest. Fifteen leaves, per variety, were assessed. Elasticity was measured by placing a medium-sized leaf in the palm of the hand and closing it, thereby turning the leaf back on itself and simulating mechanical stress. The elasticity of leaves was graded based on the following scale:

1 Completely resumes the original shape without damage
2 Resumes original shape with damage covering up to 5% leaf area
3 Resumes original shape with damage covering up to 10% leaf area
4 Does not resume original shape with damage covering up to 10% leaf area
5 Does not resume original shape with damage covering >10% leaf area

4.4.2 Leaf shape

A visual description of leaves was also conducted to illustrate differences between varieties. The shape of leaves was classified using the leaf-shape guide proposed by Swink and Wilhem (1994) and Appendix 1.

4.4.3 Degree of savoyness

The degree of savoyness, or leaf puckering, of babyleaf spinach varieties was determined using the following scale:

1 Completely flat, no leaf puckering observed
2 Small leaf puckering covering up to 20% leaf area
3 Leaf puckering covering up to 40% leaf area
4 Leaf puckering covering up to 60% leaf area
5 Leaf puckering covering >60% leaf area

Medium sized leaves representing an average sample from each variety were used for this assessment. For practical reasons the numbering scale was further grouped into three classes: a score of 1 = flat, 2 to 3 = semi-savoy, and 4 to 5 = savoy (Appendix 2).

4.4.4 Degree of leaf cupping

The degree of leaf cupping in babyleaf spinach varieties was determined using the following scale:

1 Flat leaf margins
2 Slight curving of leaf margins only
3 Leaf margins curved and extended up to 1cm inside
4 Leaf margins curved and extended up to half-way towards mid rib

Medium sized leaves representing an average sample were used for this assessment. An example of the scale used is shown in Appendix 3.

4.4.5 Determination of K and NO_3^-

Approximately 10 g of fresh leaves were ground in 40 mL of Millipore water (MQ). Samples were centrifuged at 3000 rpm for 5 min. The measurement of K in leaves was determined using a Merck K test kit (product # 1.10042.0001). The reaction zone of the test stripe was immersed in sample extract for 1 sec and then placed into the reagent (K1) for 1 min. The colour of the reaction zone then was compared to the closest colour field provided to determine mg L^{-1} of K. A similar procedure was followed to determine leaf NO_3^- concentration using a Merck Nitrate test kit (product # 1.10020.0001), but after the reaction zone of the test strip was immersed in sample extract the corresponding concentration was immediately determined using the colour field provided.

4.5 Extraction of chlorophyll

The measurement of chlorophyll in the leaves of varieties was determined as described by Yue et al. (2009), with some modifications. Approximately 10 mg of fresh leaf tissue was transferred into a micro test tube, after which 1.4 ml of 95% (v/v) ethanol was added. Samples were incubated in a water bath overnight at 60°C. Extracts were analysed at 645 and 663 nm on a spectrophotometer (Beckman Coulter DU800, USA); with specific absorption coefficients determined according to MacKinney (1941). The individual and total chlorophyll concentration of leaves was determined using the following formulas:

$$\text{Chlorophyll a} = 12.72\ (A_{663}) - 2.59\ (A_{645})$$
$$\text{Chlorophyll b} = 22.88\ (A_{645}) - 4.68\ (A_{663})$$
$$\text{Total chlorophyll} = 20.29\ (A_{645}) + 8.02\ (A_{663})$$
$$\text{Chlorophyll (mg g}^{-1}\text{)} = \frac{\text{chlorophyll (mg L}^{-1}\text{)} \times 0.001\ \text{(L)}}{\text{Fresh weight (g)}}$$

4.6 Leaf colour and respiration

A sample of medium sized leaves was used for leaf colour and respiration. Leaf colour was measured using a Minolta SPADF colour meter for the initial sample as well as 7 days after harvest (DAH). The respiration rate of leaves at ambient temperature and a storage temperature of 5°C was also measured 4 DAH using 40 g of fully expanded leaf sample. Gas samples were analysed using a GOW-MAC (Pennsylvania, USA) series 580 gas chromatography instrument.

4.7 Extraction of non-structural carbohydrates

4.7.1 Sample preparation and extraction

The measurement of glucose, fructose, sucrose and starch was determined as described by Cheng et al., (2004) and Ranwala and Miller (2009), with some modifications. In brief, a 50 mg tissue sample was placed into a 10 mL centrifuge tube. The soluble sugars such as glucose, fructose and sucrose were extracted by adding 3 mL of 80% (v/v) ethanol and incubating at 70°C for 1 h. Samples were then centrifuged at 6000 rpm for 10 min, the supernatant was collected. Two more extractions were conducted, the same as above, with the supernatant being combined. Mannitol was used as an internal standard by adding 0.5 mL of 1 mg ml^{-1} mannitol in 80% ethanol to the combined extract. The extract was then passed through 1 mL of Amberlite (Sigma, A9960) and 1 mL of Dowex 50W (Sigma, 217468) ion exchange resin. Extracts were than evaporated overnight at 55°C, by taking 1.5 mL in the eppendorf tubes and placing in a rotary evaporator (Univapo, Germany). The dried extract was then hydrated by dissolving with 1 mL of MQ water. Samples were then passed through a 0.45 µm filter and placed into vials for analysis. The amount of glucose, fructose and sucrose were calculated by using calibration curves from standard sugars and expressed on a fresh weight basis.

After soluble sugar extraction, the solid residue was dried overnight at 55°C for starch analysis. To the dried samples, 2 mL of Na-acetate buffer (100 mmol, pH 4.5) was added and placed into boiling water for 30 min. The residue was then cooled, and added 50 units of amyloglucosidase (in 1 mL of above Na-acetate buffer) to each sample tube. The sample tubes were then placed in a water bath for 48 h at 55°C, to convert starch to glucose. The digest was then cooled and centrifuged at 6000 rpm for 10 min at room temperature. The supernatant was then cooled and filtered to 0.45 µm and placed into vials for analysis. The amount of starch was estimated by calculating the amount of glucose comparing with a standard curve derived from standard glucose.

4.7.2 Quantification and HPLC conditions

Samples were analysed using a Dionex ASI-100 high-pressure liquid chromatography (HPLC) instrument fitted with a high-performance anion exchange chromatography with pulse amperometric detection (HPAE-PAD). The column used for separation was a Dionex CarboPac™ (4 × 250 mm, part # 035391). The injection volume per sample was 25 µl, with a mobile phase of 200 mmol NaOH at a flow rate of 1 mL min^{-1}.

4.8 Stomatal density and cell size

The stomatal density and size of epidermal cells were measured using leaf cell imprints. Imprints were made by painting the adaxial surface of leaves with clear nail varnish (OPI, USA). The imprint was then peeled away from the leaf surface using transparent adhesive tape. Three imprints were made per variety. Images of leaf cell structure were captured using a microscope (Leica DM2500M, Germany), fitted with a digital camera (Leica DFC500, Germany). Images were then analysed using interpretation software (Leica application suite version 3.7).

The number of stomata in 0.2 mm^2 was counted. The epidermal cell areas of 10 cells closest to the centre of the selected area were then measured using the Leica software (Image 1).

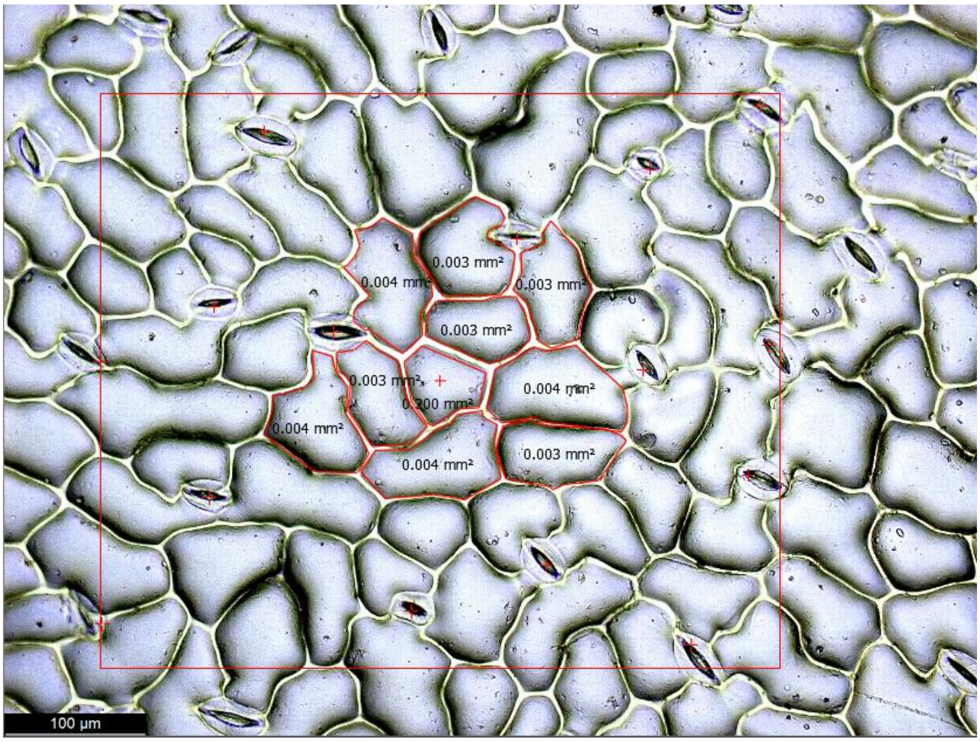


Image 1: An example of the images captured used to asses the average cell size and stomal density of differnt varieties of babyleaf spinach. The oval shapes with dark centers are stomata, and other shapes are epidermal leaf cells.

4.9 Shelf life storage conditions and assessment criteria

After harvest, three leaf samples, each about 1 kg, were taken from individual varieties for shelf life experiments. From this initial sample 100 g of fresh leaves either unprocessed (without washing) or processed (washed and spin dried), were assessed for either: undamaged, broken, bruised, split, wilted or tearing at the apex by recording the number and weight of leaves in these groups. After visual assessment leaves were placed in low-density plastic bags and double-folded for storage at 5°C (Labec laboratory equipment Pty. Ltd). A further assessment using the above criteria was performed 7 days after harvest (DAH). Ongoing assessment of samples occurred daily after 7 DAH, with samples being visual examined for signs of leaf deterioration, microbial breakdown and desiccation. Samples were observed for a turning point of their freshness to a pale colouration or any deterioration and were considered to fail visual shelf life criteria when they exhibited signs of these forms of loss of quality, and were hence rendered unsalable.

4.10 Rocket shelf life experiments

The visual quality of European wild rocket and arugula leaves was assessed over a range of different postharvest storage temperatures. The visual quality of leaves was assessed after harvest during storage at 0, 4, and 7ºC. The shelf life of leaves from respective species was also examined when grown during a range of different seasonal conditions and harvest numbers. The shelf life of leaves grown under different levels of applied nitrogen and stored at 0°C was also determined.

The leaves for individual shelf life experiments were sourced from field experiments conducted at Ellis Lane, New South Wales, Australia, latitude 33° 55’ and longitude 150° 58’. Three different cultivars were used to represent respective rocket species (Table 3). Leaves were sampled from field experiments from either a completely randomised block design (European wild rocket) or an incomplete block design (arugula), both of which had four blocks. A sample of leaves weighing approximately 300 g was taken from individual replicates.

Rocket leaves were sampled from field nitrogen experiments to which four rates of nitrogen were applied (0, 50, 150, and 250 Kg N ha^{-1}). Field nitrogen experiments for both European wild rocket and arugula were a completely randomised block design consisting of four blocks.

Table 3: The source of seeds for each species cultivar used within shelf life experiments for European wild rocket (*Diplotaxis tenuifolia* (L.) DC. (L.) DC.) and arugula (*Eruca sativa* Mill.).

Code	Species	Cultivar	Batch number	Commercial seed supplier
DT1	*D. tenuifolia*	EWR	ST06-2040 V59	Lefroy valley
DT2	*D. tenuifolia*	Apollo	BMP7206262	Fairbanks seeds
DT3	*D. tenuifolia*	Nature	786755	Seminis Ltd
ES4	*E. sativa*	Cultivated	AZW0806133	Fairbanks seeds
ES5	*E. sativa*	Highway	BHP0806228	Fairbanks seeds
ES6	*E. sativa*	Myway	BHQ0806227	Fairbanks seeds

*European wild rocket.

Thirty fully expanded leaves measuring >10 cm in length per replicate were randomly chosen from field experiments. Leaves were then transferred into perforated plastic bags and placed in dark controlled temperature chambers (Labec Laboratory Equipment Pty. Ltd., model ICCBOD), each set at a constant temperature of 0, 4, or 7°C.

During storage at different temperatures leaves of European wild rocket and arugula were inspected; individual leaves within temperature replicates were considered to either pass or fail visual quality criteria. The criteria used to determine if leaves were acceptable for consumption were signs of chlorosis, wilting, and microbial spoilage (Table 4). The leaves that passed these visual quality criteria were placed back into the perforated bags and returned to their storage treatment until the next sampling event.

Table 4: The visual shelf life quality criteria used to assess whether European wild rocket (*Diplotaxis tenuifolia* (L.) DC. (L.) DC.) and arugula (*Eruca sativa* Mill.) leaves pass or fail shelf life.

Quality characteristic	**Pass**	**Fail**
Chlorosis/yellowing	>80% of leaf area green	<80% of leaf area green
Wilting	No signs of wilting	Leaf show signs of wilting
Microbial spoilage	Not present	Present

Wild rocket and arugula leaves were sampled throughout storage at different postharvest temperatures. The visual inspection of leaves occurred at 5 and 10 DAH; after which leaves were sampled at different intervals according to the rate of decline in shelf life, which was not consistent between species, season, or storage temperature. The point at which shelf life was considered to have failed the visual quality criteria was when >50% of the leaves had failed, or when >15 leaves from individual replicates had failed.

The analysis of data was conducted using the statistical package GenStat® (12th edition, VSN International Ltd., United Kingdom). Significant differences were considered when $P<0.05$, and when the difference between treatment means was greater than that of the least significant difference (5%). The shelf life data for seasonal and multi-harvest experiments was analysed using the analysis of variance (ANOVA) completely randomised block design for European wild rocket experiments; and the ANOVA unbalanced design for arugula experiments.

All shelf life data for nitrogen experiments was analysed using the ANOVA completely randomised block design. The rate at which leaves failed visual quality criteria was analysed using quadratic polynomial regression. A significant interaction between variables within the regression model was considered when $P<0.05$. The shelf life value within regression models was calculated by back transforming the polynomial equation ± the accuracy of the predicted model. Data was checked for normality, and transformed where required.

5 Results

5.1 Study tour to the Rijk Zwaan breeding institute in Holland.

The first activity of the project was a trip by the project leader to the Rijk Zwaan breeding institute in Holland. The purpose was to meet with breeders and fine-tune the experimental protocol and variables to be measured to ensure compatible methodologies were used, and to discuss the experimental questions that would be answered by the project. The main outcomes from these discussions are summarised below, and a detailed report is attached as appendix 1.

- Omit the controlled growth study and focus on 4 field studies.
- Field studies to be based on a range of varieties known to provide a range in shelf life, and grow over different seasons.
- Expand the list of variables measured from field trials.
- Include a processing study with each field study.
- Only undertake nutrition and density studies if resources permit.
- Focus on spinach as this is a more significant crop than rocket.

The results from individual experiments can be grouped into those that have a positive correlation with shelf life or those that have a negative correlation with shelf life. The factors that had a positive correlation with shelf life across experiments include: leaf colour at harvest, leaf colour 7 DAH and leaf thickness (Table 5). This means leaves of a darker green and hence with more chlorophyll, have a longer shelf life than leaves with a lower colour reading. Similarly, leaves that are thicker have a longer shelf life than those that are thinner. These results illustrate that in the selection of varieties for commercial production, leaf colour and leaf thickness should be considerations if superior shelf life is desired.

The factors that had a negative correlation with shelf life include specific leaf area (SLA), respiration rate, chlorophyll b, plant height, plant weight and leaf area (Table 5). Four of these factors relate to the growth stage of plants and indicate that smaller leaves are able to receive more mechanical damage than larger leaves before visible signs of reduced quality are observed. Additionally, a leaf with a higher respiration rate fails shelf life more quickly than one with a lower respiration rate. This makes sense as stored reserves and water are being lost more quickly from the leaf with a higher rate of respiration. The rocket shelf life results are presented separately.

Table 5: Summary of the type of correlation and factors that influence the shelf life of either unwashed or washed babyleaf spinach leaves.

Positive correlation	Negative correlation
Leaf colour at harvest	SLA
Leaf colour 7 DAH	Respiration at 5 and 20 °C
Leaf thickness	Chlorophyll b
	Plant height
	Plant weight
	Leaf area

5.2 Spinach Experiment 1

There were two correlations identified from experiment 1 between the shelf life of leaves and factors measured. The correlation between leaf colour at harvest and shelf life of washed leaves was 0.39; while correlation between leaf colour 7 DAH and shelf life of washed leaves was 0.32 (Table 6). This response was influenced by the longer shelf life of the variety Toucan. If data for this variety is removed from analysis then there is no correlation between leaf colour at harvest or leaf colour 7 DAH and shelf life of washed leaves.

There was also strong correlation between growth factors such as: SLA, leaf dry matter content (LDMC) and dry matter % and variety (Table 6). These correlations were likely influenced by the developmental stage of respective varieties.

Table 6: Summary of the factors that influence the shelf life of babyleaf spinach varieties.

Rank	Order [a]	Factor 1	Factor 2	Correlation
1	2	SLA	Variety	0.54
2	3	LDMC	Variety	-0.41
3	3	Dry matter %	Variety	-0.41
4	**3**	**Leaf colour at harvest**	**Shelf life washed**	**0.39**
5	3	Shelf life unwashed	Variety	-0.33
6	**3**	**Leaf colour 7DAH**	**Shelf life washed**	**0.32**

[a] Order 2, correlation between ± 0.50 and 0.75; order 3, correlation between ± 0.25 and 0.50.

Order 3 correlations for shelf life

A correlation between leaf colour at harvest and 7 DAH was identified with the shelf life of washed leaves. However, this response was influenced by the longer shelf life of the variety Toucan in both instances (Figures 1 and 2).

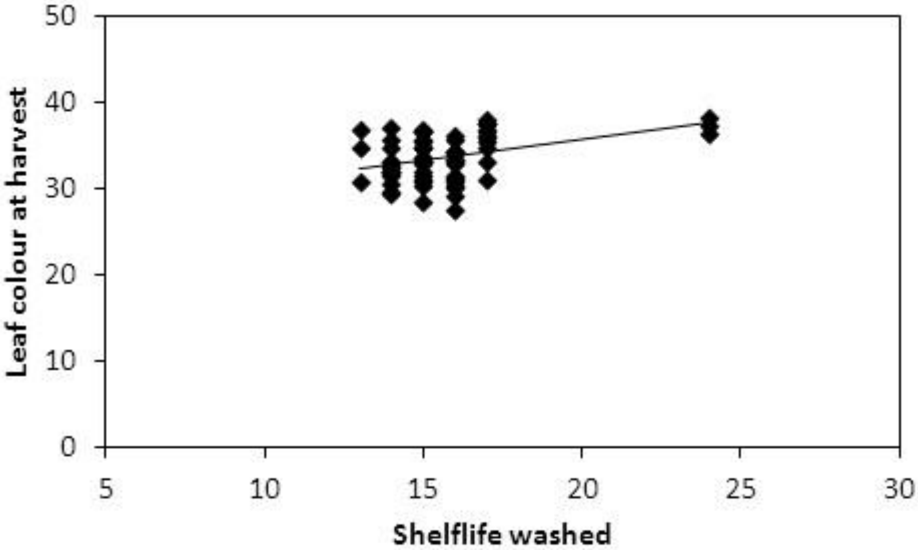


Figure 1: Relationship between leaf colour at harvest and shelf life of washed babyleaf spinach.

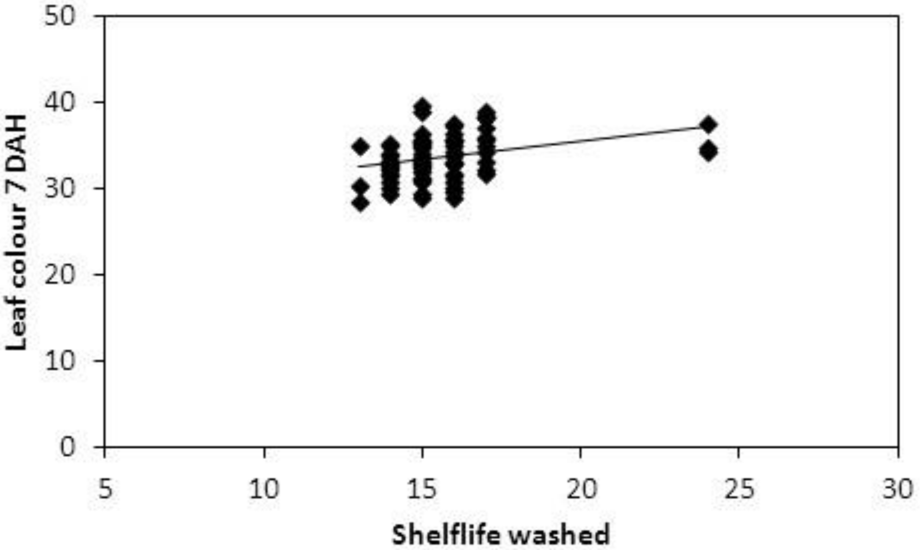


Figure 2: Relationship between leaf colour 7 days after harvest and shelf life of washed babyleaf spinach.

Table 7: Correlation matrix between variables measured and shelf life for experiment 1.

🟦 Order 2, correla
🟥 Order 3, correla

	Variety	Shelf life commercially	Shelf life	Shelf life unwashed	Leaf colour	Leaf colour	SLA	LDMC	Dry matter	Harvest undan commercially w
Variety	-									
Shelf life commercially	-0.11	-								
Shelf life washed	-0.17	🟦 0.29	-							
Shelf life unwashed	🟦 -0.33	🟦 0.33	🟦 0.49	-						
Leaf colour harvest	-0.07	0.06	🟦 0.39	-0.05	-					
Leaf colour 7DAH	0.08	0.03	🟦 0.32	-0.11	0.34	-				
SLA	🟥 0.54	-0.03	-0.08	-0.05	-0.24	0.10	-			
LDMC	🟦 -0.41	0.15	0.11	0.16	0.11	-0.18	-	-		
Dry matter %	🟦 -0.41	0.15	0.11	0.16	0.11	-0.18	-	1.00	-	
Harvest undamaged	0.05	-0.10	🟥 0.53	0.14	0.27	0.26	0.04	0.05	0.05	-
Harvest broken	0.01	🟦 0.28	🟦 -0.34	-0.12	-0.30	-0.19	0.02	-0.13	-0.13	-0.65
Harvest bruised	-0.08	🟦 -0.25	-0.19	-0.02	0.06	-0.06	-	0.13	0.13	-0.35
7DAH undamaged	-0.23	0.06	🟦 0.49	0.13	0.38	0.33	-	0.15	0.15	0.70
7DAH broken	🟦 0.32	-0.06	🟦 -0.46	-0.19	-0.36	-0.28	0.17	-0.22	-0.22	-0.68
7DAH bruised	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7DAH wilted	🟦 -0.40	0.01	-0.17	🟦 0.27	-0.12	-0.26	-	0.34	0.34	-0.14
Harvest undamaged	-0.24	-0.04	🟦 0.49	0.23	0.37	0.20	-	0.32	0.32	0.82
Harvest broken washed	🟦 -0.43	🟦 0.35	-0.18	0.05	-0.01	-0.19	-	0.27	0.27	-0.36
Harvest bruised washed	-0.20	-0.15	-0.03	0.08	0.05	-0.04	-	0.22	0.22	-0.18
7DAH undamaged	🟦 -0.33	0.04	🟦 0.39	0.16	0.39	0.22	-	0.32	0.32	0.64
7DAH broken washed	-0.02	0.05	🟥 -0.53	-0.11	-0.28	-0.44	-	0.24	0.24	-0.52
7DAH bruised washed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7DAH wilted washed	🟦 -0.43	0.00	-0.13	🟦 0.32	-0.08	-0.23	-	0.35	0.35	-0.04
Harvest undamaged	0.10	-0.13	🟦 0.40	0.13	0.35	0.32	0.07	-0.01	-0.01	0.60
Harvest broken	-0.23	0.12	🟦 -0.32	0.04	-0.16	-0.37	-	0.04	0.04	-0.56
Harvest bruised	🟦 0.25	0.05	-0.15	🟦 -0.29	-0.35	0.07	0.17	-0.09	-0.09	-0.07
7DAH undamaged	-0.11	-0.05	🟦 0.32	0.24	0.24	0.18	-	0.37	0.37	0.58
7DAH broken unwashed	-0.05	0.12	🟦 -0.28	-0.01	-0.28	-0.27	0.20	-0.32	-0.32	-0.59
7DAH bruised unwashed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7DAH wilted unwashed	🟦 0.39	-0.18	-0.17	🟥 -0.59	0.05	0.19	0.12	-0.17	-0.17	-0.07

	Harvest broken commercially washed	Harvest bruised commercially washed	7DAH undamaged commercially washed	7DAH broken commercially washed	7DAH bruised commercially washed
Harvest broken	-				
Harvest bruised	-0.48	-			
7DAH undamaged	-0.72	0.08	-		
7DAH broken	0.71	-0.10	-0.98	-	
7DAH bruised	0.00	0.00	0.00	0.00	-
7DAH wilted	0.07	0.08	-0.17	-0.04	0.00
Harvest	-0.75	-0.01	0.82	-0.83	0.00
Harvest broken	0.56	-0.26	-0.16	0.11	0.00
Harvest bruised	-0.45	0.76	0.25	-0.27	0.00
7DAH undamaged	-0.69	0.13	0.90	-0.91	0.00
7DAH broken	0.47	0.04	-0.64	0.61	0.00
7DAH bruised	0.00	0.00	0.00	0.00	0.00
7DAH wilted	-0.06	0.12	-0.03	-0.17	0.00
Harvest	-0.62	0.08	0.70	-0.67	0.00
Harvest broken	0.52	0.00	-0.61	0.58	0.00
Harvest bruised	0.18	-0.16	-0.17	0.19	0.00
7DAH undamaged	-0.64	0.12	0.69	-0.71	0.00
7DAH broken	0.66	-0.14	-0.67	0.67	0.00
7DAH bruised	0.00	0.00	0.00	0.00	0.00
7DAH wilted	0.06	0.02	-0.16	0.23	0.00

	7DAH wilted commercially washed	**Harvest undamaged washed**	**Harvest broken washed**	**Harvest bruised washed**	**7DAH undamaged washed**	**7DAH broken washed**	**7DAH bruised washed**	**7DAH wilted washed**
7DAH wilted	-							
Harvest	-0.02	-						
Harvest broken	0.24	-0.07	-					
Harvest bruised	0.10	0.24	-0.05	-				
7DAH undamaged	-0.02	0.91	0.05	0.30	-			
7DAH broken	0.21	-0.36	0.53	-0.07	-0.34	-		
7DAH bruised	0.00	0.00	0.00	0.00	0.00	0.00	-	
7DAH wilted	0.97	0.11	0.23	0.16	0.12	0.17	0.00	-
Harvest	-0.16	0.61	-0.27	0.18	0.61	-0.46	0.00	-0.04
Harvest broken	0.20	-0.54	0.23	-0.15	-0.53	0.41	0.00	0.08
Harvest bruised	-0.08	-0.14	0.05	-0.07	-0.17	0.06	0.00	-0.09
7DAH undamaged	0.08	0.73	-0.07	0.28	0.71	-0.31	0.00	0.22
7DAH broken	0.05	-0.69	0.13	-0.25	-0.69	0.25	0.00	-0.10
7DAH bruised	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7DAH wilted	-0.34	-0.20	-0.12	-0.12	-0.18	0.20	0.00	-0.34

	Harvest undamaged unwashed	**Harvest broken**	**Harvest bruised**	**7DAH undamaged unwashed**	**7DAH broken**	**7DAH bruised**	**7DAH wilted unwashed**
Harvest	-						
Harvest broken	-0.84	-					
Harvest bruised	-0.30	-0.26	-				
7DAH undamaged	0.72	-0.70	-0.07	-			
7DAH broken	-0.75	0.76	0.01	-0.92	-		
7DAH bruised	0.00	0.00	0.00	0.00	0.00	-	
7DAH wilted	-0.05	-0.04	0.15	-0.35	-0.04	0.00	-

5.3 Spinach Experiment 2

There was a third order correlation between leaf thickness and the shelf life of unwashed babyleaf spinach. The correlation between these factors was 0.29 and represents a relatively weak correlation (Table 8).

There was also a strong correlation between variety and leaf colour at harvest and 7 DAH. These correlations were both negative, meaning that faster-growing varieties have lower leaf colour than slower-growing varieties. The shelf life of washed leaves and leaf respiration were also correlated with variety (Table 8).

Table 8: Summary of the factors that influence the shelf life of babyleaf spinach varieties.

Rank	Order [a]	Factor 1	Factor 2	Correlation
1	2	Leaf colour 7 DAH	Variety	-0.71
2	2	Leaf colour harvest	Variety	-0.69
3	3	Shelf life washed	Variety	-0.30
4	3	Respiration	Variety	-0.29
5	**3**	**Leaf thickness**	**Shelf life unwashed**	**0.29**

[a] Order 2, correlation between ± 0.50 and 0.75; order 3, correlation between ± 0.25 and 0.50.

Order 3 correlations for shelf life

There was a correlation between the leaf thickness and shelf life of unwashed leaves of baby spinach (Figure 3). The correlation was positive and hence a thicker leaf generally achieved longer shelf life. There was a wide range in leaf thickness and shelf life, making a clear relationship between these factors difficult.

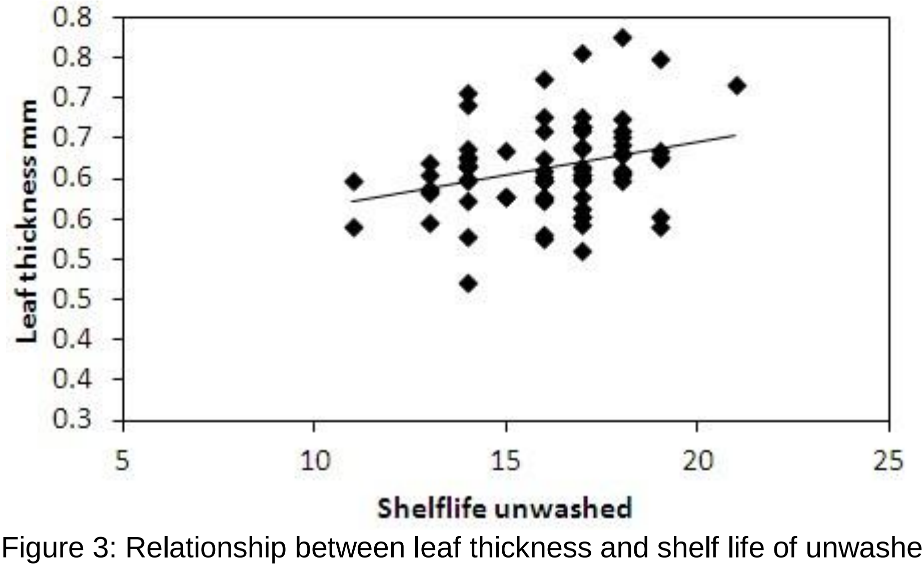


Figure 3: Relationship between leaf thickness and shelf life of unwashed babyleaf spinach.

Table 9: Correlation matrix between variables measured and shelf life for experiment 2.

🟦 Order 2, correlation
🟥 Order 3, correlation

	Variety	Shelf life	Shelf life unwashed	Respiration	Leaf colour	Leaf colour	Leaf thickness	Harvest undamaged	Harvest broken	Harvest bruised washed
Variety	-									
Shelf life	-0.30	-								
Shelf life	0.21	0.29	-							
Respiration	-0.29	-0.01	-0.08	-						
Leaf colour	-0.69	0.15	-0.21	0.12	-					
Leaf colour 7	-0.71	0.18	-0.19	0.12	0.93	-				
Leaf thickness	0.12	0.03	0.29	0.01	0.02	0.01	-			
Harvest	-0.21	0.20	0.00	0.08	0.18	0.10	0.19	-		
Harvest broken	0.05	-0.16	-0.11	0.05	-0.03	0.03	-0.18	-0.88	-	
Harvest bruised	0.44	-0.12	0.12	-0.24	-0.37	-0.32	0.02	-0.24	-0.18	-
Harvest split	-0.07	0.03	0.25	-0.15	-0.04	0.01	-0.07	-0.10	-0.19	0.08
7DAH	-0.34	0.10	-0.20	0.00	0.45	0.39	0.05	0.61	-0.35	-0.54
7DAH broken	0.14	0.02	0.05	-0.04	-0.33	-0.28	-0.16	-0.46	0.27	0.43
7DAHbruised	-0.41	0.23	-0.03	0.05	0.31	0.31	-0.10	0.15	-0.14	-0.19
7DAH wilted	0.46	-0.28	0.28	0.05	-0.29	-0.27	0.23	-0.32	0.19	0.25
Harvest	-0.26	0.10	0.03	0.27	0.19	0.14	0.25	0.46	-0.30	-0.41
Harvest broken	-0.17	-0.15	-0.06	0.11	0.05	0.10	-0.15	-0.16	0.09	0.05
Harvest bruised	-0.12	-0.01	-0.15	-0.08	0.10	0.13	0.16	0.05	-0.03	-0.05
Harvest split	0.35	-0.01	0.02	-0.29	-0.21	-0.20	-0.18	-0.34	0.23	0.35
7DAH	0.31	-0.01	0.02	-0.24	-0.16	-0.16	-0.16	-0.33	0.27	0.28
7DAHbroken	0.21	-0.17	0.05	-0.03	-0.17	-0.13	0.03	-0.19	0.05	0.09
7DAH bruised	0.32	0.02	-0.02	-0.30	-0.19	-0.18	-0.18	-0.27	0.14	0.41
7DAH wilted	0.39	-0.06	0.02	-0.25	-0.23	-0.22	-0.15	-0.38	0.29	0.32

Harvest split	-							
7DAH	-0.16	-						
7DAH broken	0.05	-0.86	-					
7DAHbruised	0.27	0.23	-0.25	-				
7DAH wilted	0.13	-0.33	-0.19	-0.19	-			
Harvest	0.02	0.34	-0.29	0.06	-0.10	-		
Harvest broken	0.18	-0.09	0.14	0.03	-0.10	-0.12	-	
Harvest bruised	0.01	-0.06	0.08	-0.01	-0.03	0.11	-0.29	-
Harvest split	-0.11	-0.25	0.18	-0.07	0.15	-0.86	-0.38	-0.08
7DAH	-0.21	-0.20	0.08	-0.08	0.24	-0.75	-0.43	-0.09
7DAHbroken	0.42	-0.24	0.15	-0.02	0.18	0.13	0.50	-0.03
7DAH bruised	-0.13	-0.29	0.19	-0.04	0.20	-0.78	-0.31	-0.10
7DAH wilted	-0.15	-0.24	0.13	-0.10	0.23	-0.82	-0.39	-0.09
	Harvest split washed	**7DAH undamaged washed**	**7DAH broken washed**	**7DAH bruised washed**	**7DAH wilted washed**	**Harvest undamaged unwashed**	**Harvest broken unwashed**	**Harvest bruised unwashed**

Harvest split	-				
7DAH	0.92	-			
7DAHbroken	-0.38	-0.49	-		
7DAH bruised	0.89	0.90	-0.36	-	
7DAH wilted	0.97	0.96	-0.38	0.85	-
	Harvest split unwashed	**7DAH undamaged unwashed**	**7DAH broken unwashed**	**7DAH bruised unwashed**	**7DAH wilted unwashed**

5.4 Spinach Experiment 3

There were second-order correlations between the shelf life of washed leaves and leaf colour at harvest and 7 DAH and the SLA (Table 10). The leaf colour correlations were both positive and agree with results from other experiments that greener leaves have longer shelf life. The correlation between shelf life of washed leaves and SLA was negative, meaning that larger leaves have a shorter shelf life than smaller leaves, again confirming results from other experiments.

Factors that were correlated with the shelf life of unwashed leaves include: SLA, leaf respiration at 5 and 20°C, leaf colour 7 DAH and chlorophyll b (Table 10). Many of these factors were also correlated with variety. Leaf respiration and chlorophyll b had a negative correlation with shelf life.

Table 10: Summary of the factors that influence the shelf life of babyleaf spinach varieties.

Rank	Order [a]	Factor 1	Factor 2	Correlation
1	**2**	**Leaf colour 7 DAH**	**Shelf life washed**	**0.69**
2	2	Shelf life unwashed	Variety	0.62
3	**2**	**SLA**	**Shelf life washed**	**-0.61**
4	2	Respiration 5 °C	Variety	-0.61
5	**2**	**SLA**	**Shelf life unwashed**	**-0.56**
6	**2**	**Leaf colour at harvest**	**Shelf life washed**	**0.56**
7	2	Chlorophyll a	Variety	-0.56
8	**2**	**Respiration 20 °C**	**Shelf life unwashed**	**-0.52**
9	**2**	**Leaf colour 7 DAH**	**Shelf life unwashed**	**0.51**
10	**2**	**Respiration 5 °C**	**Shelf life unwashed**	**-0.50**
11	2	Elasticity	Variety	0.50
12	**2**	**Chlorophyll b**	**Shelf life unwashed**	**-0.50**
13	2	Total Chlorophyll weight	Variety	-0.54

[a] Order 2, correlation between ± 0.50 and 0.75.

Order 2 correlations for shelf life

The colour of leaves 7 DAH was positively correlated with the shelf life of washed leaves (Figure 4). This response was, however, influenced by the longer shelf life of the variety Turtle. If this variety is removed from analysis there is no longer a correlation between these factors.

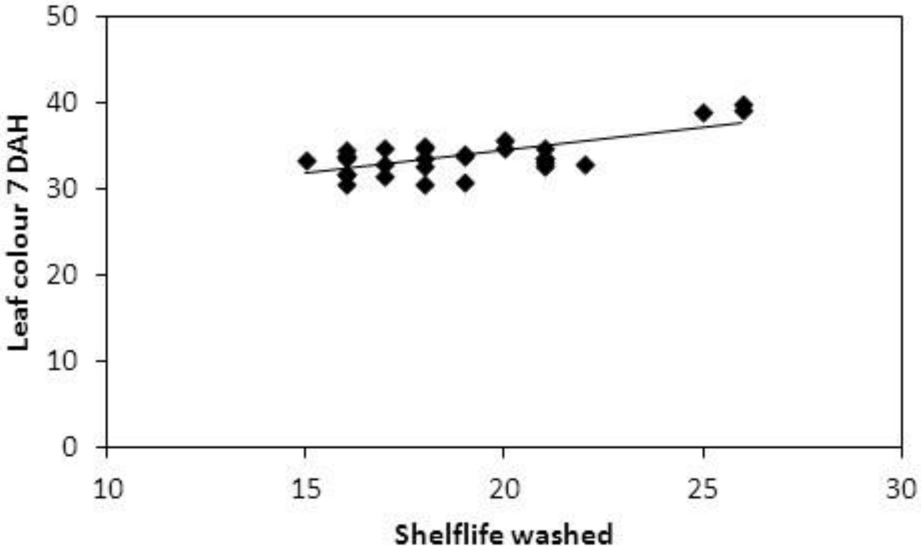


Figure 4: Relationship between leaf colour 7 DAH and shelf life of washed babyleaf spinach.

A similar response was shown for leaf colour at harvest. If Turtle is removed from analysis then there is no correlation between factors (Figure 5).

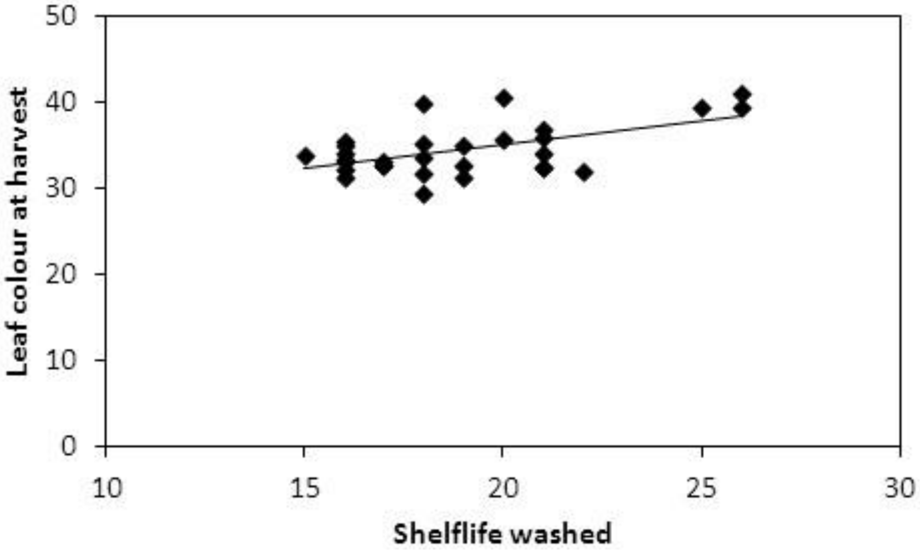


Figure 5: Relationship between leaf colour at harvest and shelf life of washed babyleaf spinach.

The relationship between SLA and shelf life of washed leaves was also influenced by the longer shelf life of the variety Turtle (Figure 6). When this variety is removed from analysis there is no correlation between these factors.

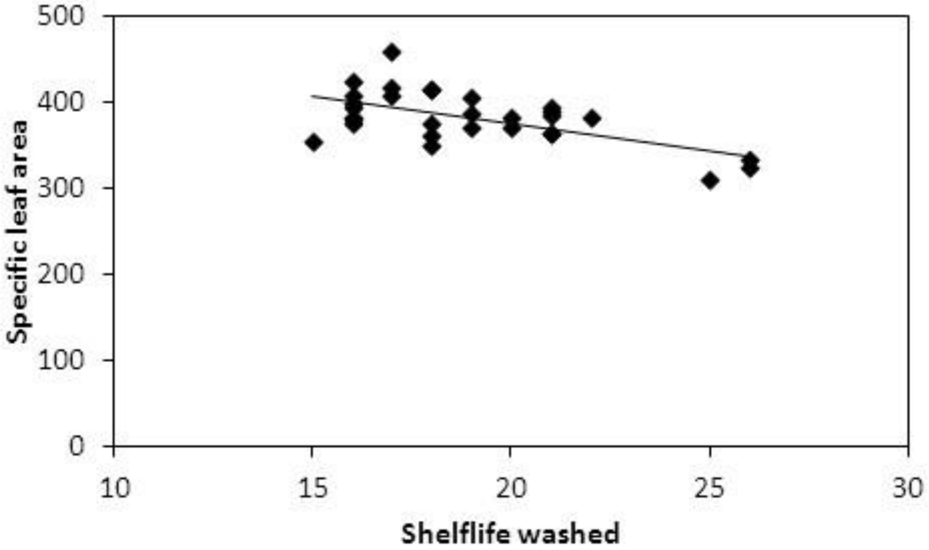


Figure 6: Relationship between specific leaf area and shelf life of washed babyleaf spinach.

There was a negative correlation between SLA and shelf life of unwashed leaves (Figure 7). This means that leaves from smaller plants achieved a longer shelf life than leaves from larger heavier plants.

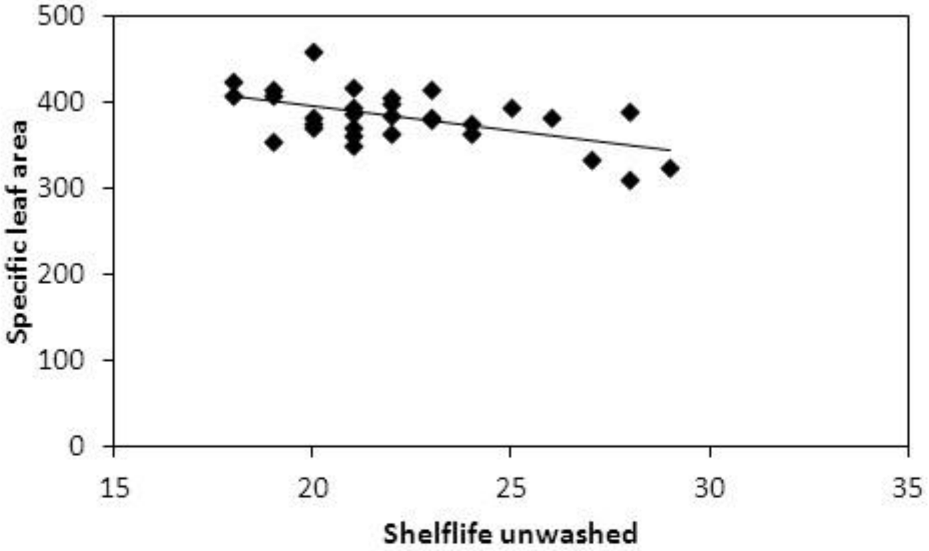


Figure 7: Relationship between specific leaf area and shelf life of unwashed babyleaf spinach.

Higher levels of leaf respiration during storage resulted in reduced shelf life, while lower respiration resulted in longer shelf life (Figure 8). This response was similar to that identified in separate experiments.

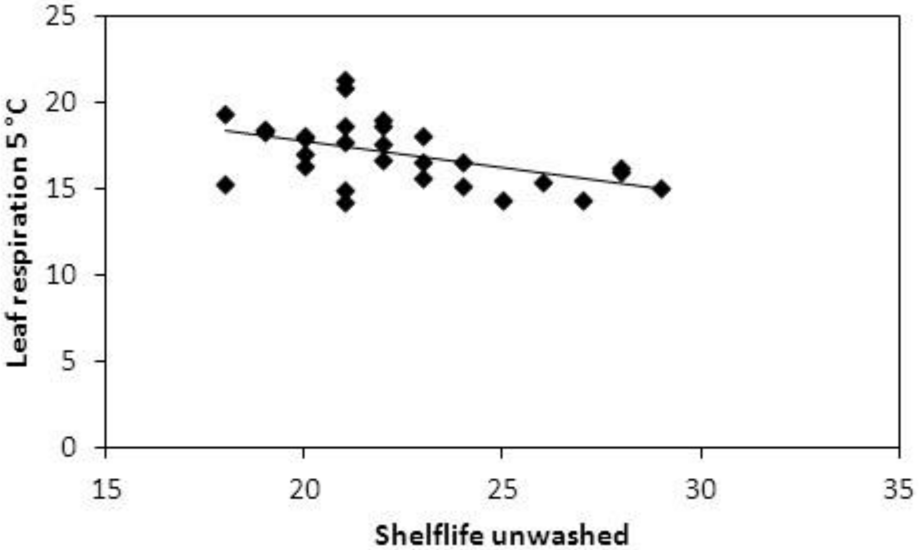


Figure 8: Relationship between leaf respiration at 5°C and shelf life of unwashed babyleaf spinach.

Leaf respiration at 20°C followed a similar trend to respiration at 5°C, with higher levels of leaf respiration resulting in reduced shelf life (Figure 9).

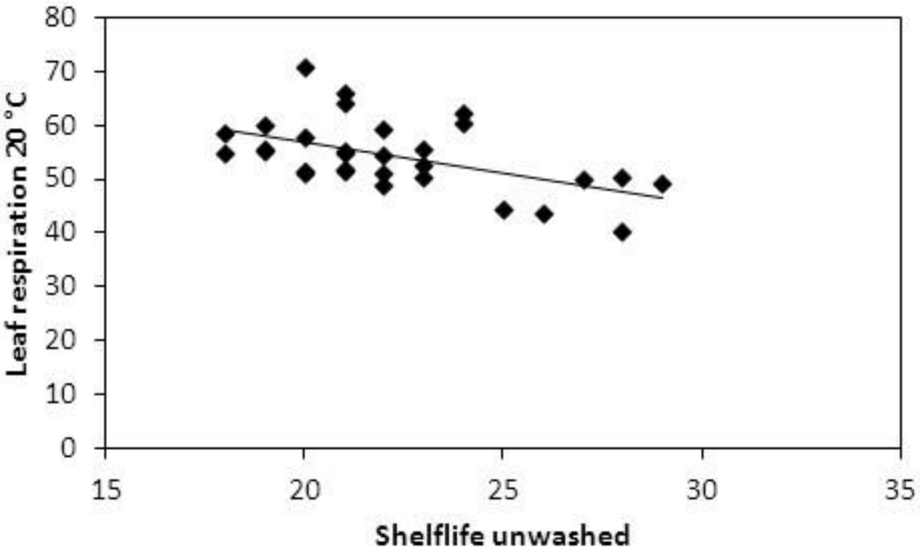


Figure 9: Relationship between leaf respiration at 20°C and shelf life of unwashed babyleaf spinach.

The colour of leaves 7 DAH was positively correlated with the shelf life of unwashed leaves (Figure 10). This is in agreement with other experiments where darker leaves visually last for a longer period of time during storage.

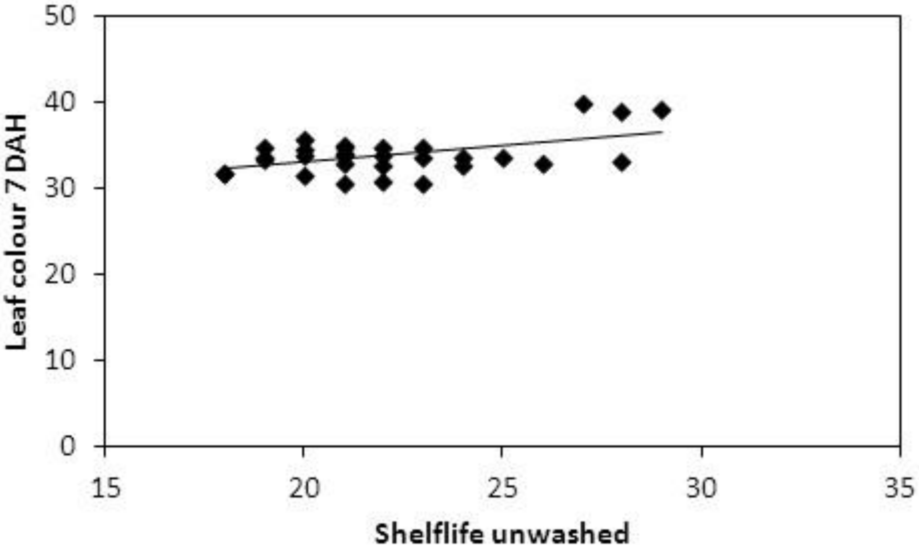


Figure 10: Relationship between leaf colour 7 days after harvest and shelf life of unwashed babyleaf spinach.

There was a negative correlation between chlorophyll b and the shelf life of unwashed leaves (Figure 11). The reason for this is unclear.

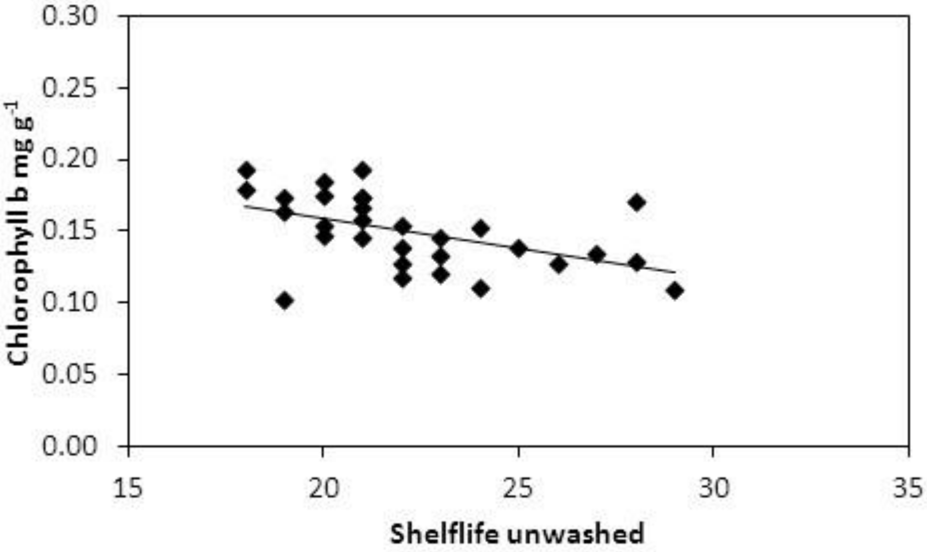


Figure 11: Relationship between chlorophyll b and shelf life of unwashed babyleaf spinach.

Table 11: Correlation matrix between variables measured and shelf life for experiment 3.

🟥 Order 2,
🟦 Order 3,

	Variety	Shelf life unwashed	Shelf life	Elasticity	Plant height	Respiration 5 °C	Respiration 20 °C	Glucose	Sucrose	Starch	Total n structural
Variety	-										
Shelf life	0.62	-									
Shelf life	0.38	0.82	-								
Elasticity	0.50	0.15	0.05	-							
Plant height	-0.36	-0.38	-0.34	-0.35	-						
Respiration 5	-0.61	-0.50	-0.42	-0.41	0.39	-					
Respiration	-0.46	-0.52	-0.37	-0.25	-0.28	0.46	-				
Glucose	-0.20	-0.18	-0.07	-0.43	0.05	0.12	0.38	-			
Sucrose	-0.13	-0.05	0.10	-0.50	0.05	0.03	0.19	0.68	-		
Starch	0.06	0.01	0.14	-0.33	0.02	-0.01	0.27	0.19	0.18	-	
Total non-	-0.15	-0.10	0.06	-0.55	0.05	0.08	0.32	0.87	0.94	0.35	-
Leaf colour at	-0.04	0.34	0.56	0.08	-0.65	-0.14	0.25	0.04	0.18	0.14	0.15
Leaf colour	0.03	0.51	0.69	-0.09	-0.48	-0.21	0.01	0.13	0.16	0.13	0.17
Chlorophyll a	-0.56	-0.47	-0.44	-0.06	-0.01	0.31	0.35	0.11	-0.06	-0.09	-0.01
Chlorophyll b	-0.48	-0.50	-0.48	0.02	-0.02	0.31	0.38	0.11	-0.07	-0.04	-0.01
Total	-0.54	-0.48	-0.45	-0.04	-0.01	0.31	0.36	0.11	-0.06	-0.08	-0.01
Total	0.14	0.35	0.38	0.07	-0.36	-0.08	0.15	0.05	-0.01	0.19	0.05
K	0.10	0.29	0.29	-0.25	0.10	-0.16	-0.17	0.02	0.15	0.19	0.13
NO3	-0.02	-0.01	-0.12	-0.16	0.26	-0.11	-0.32	-0.06	-0.09	-0.30	-0.13
Plant weight	0.35	0.05	-0.02	0.23	0.35	-0.15	-0.41	-0.26	-0.36	0.11	-0.32
Leaf	0.13	0.24	0.41	0.13	-0.11	-0.34	-0.29	-0.25	-0.28	0.05	-0.27
Leaf area	0.21	-0.08	-0.12	0.16	0.48	-0.05	-0.35	-0.19	-0.30	0.15	-0.24
SLA	-0.14	-0.56	-0.61	-0.09	0.53	0.32	0.03	0.12	0.07	-0.14	0.08
Stomatal	0.09	0.20	0.32	-0.05	-0.27	0.02	0.19	-0.06	-0.03	0.12	-0.02
Epidermal cell	0.06	0.07	-0.06	0.25	0.06	-0.08	-0.26	0.03	0.08	-0.16	0.04
Harvest	-0.29	0.02	0.33	-0.04	0.14	0.06	0.03	0.09	0.01	-0.14	0.01
Harvest	0.38	0.11	-0.08	0.13	0.14	-0.28	-0.25	-0.06	0.02	0.30	0.04
Harvest	-0.14	-0.22	-0.09	-0.18	0.04	0.02	0.18	0.00	0.18	0.46	0.19
Harvest split	0.14	-0.07	-0.37	-0.13	-0.26	0.04	0.15	0.02	0.13	0.07	0.11
Harvest apex	-0.05	0.13	0.18	0.24	0.04	-0.01	-0.26	-0.15	-0.35	-0.48	-0.36
7DAH	-0.31	0.03	0.30	-0.25	0.12	0.15	0.21	0.17	0.22	0.28	0.24
7DAH broken	0.48	0.21	0.20	0.12	0.10	-0.37	-0.30	0.17	0.18	0.34	0.23
7DAH bruised	-0.11	-0.22	-0.19	-0.12	0.53	0.15	-0.10	0.01	-0.09	0.23	-0.02
7DAH split	0.20	0.04	-0.30	0.14	-0.15	-0.14	-0.17	-0.16	-0.18	-0.30	-0.23
7DAH wilted	-0.01	-0.14	0.09	0.10	-0.11	-0.06	0.00	-0.03	0.07	-0.13	0.02
Harvest	-0.39	-0.01	0.32	-0.16	-0.13	0.11	0.30	0.21	0.13	0.16	0.18
Harvest	0.42	0.09	0.11	0.19	0.43	-0.15	-0.43	-0.24	-0.04	0.27	-0.08
Harvest	0.14	0.06	-0.09	-0.29	0.25	0.02	-0.29	0.09	0.25	0.25	0.24
Harvest split	0.15	-0.09	-0.29	-0.03	0.05	-0.13	-0.17	-0.01	0.16	-0.16	0.07
Harvest apex	0.22	0.03	-0.26	0.21	-0.14	-0.09	-0.12	-0.19	-0.23	-0.41	-0.28
7DAH	-0.27	0.05	0.36	-0.09	-0.14	0.06	0.26	0.08	0.10	0.23	0.12
7DAH broken	0.21	-0.03	0.00	-0.11	0.29	-0.14	-0.08	-0.08	0.18	0.36	0.13
7DAH bruised	0.24	0.24	0.17	0.09	0.10	-0.21	-0.39	0.12	0.11	0.12	0.14
7DAH split	0.16	-0.06	-0.34	0.14	0.05	-0.08	-0.30	-0.11	-0.08	-0.46	-0.16
7DAH wilted	0.07	-0.12	-0.17	-0.20	-0.04	0.12	0.43	0.27	0.03	0.37	0.19

	Leaf colour harvest	Leaf colour 7DAH	Chl a	Chl b	Total Chl (wt)	Total Chl area	K	NO3	Plant wt	Leaf thick ness	Leaf area	SL A	Stomatal density
Leaf colour at	-												
Leaf colour	0.85	-											
Chlorophyll a	0.06	-0.15	-										
Chlorophyll b	0.04	-0.21	0.99	-									
Total	0.05	-0.16	1.00	0.99	-								
Total	0.44	0.43	0.30	0.30	0.30	-							
K	0.10	0.09	0.07	0.05	0.07	0.22	-						
NO3	-0.34	-0.25	0.10	0.03	0.09	-0.31	0.21	-					
Plant weight	-0.35	-0.24	-0.40	-0.36	-0.40	-0.26	0.01	0.00	-				
Leaf thickness	0.28	0.41	-0.14	-0.16	-0.14	0.07	0.04	-	0.46	-			
Leaf area	-0.41	-0.32	-0.24	-0.19	-0.23	-0.23	0.13	0.02	0.96	0.40	-		
SLA	-0.74	-0.79	0.23	0.28	0.24	-0.39	-	0.27	0.06	-0.39	0.20	-	
Stomatal	0.36	0.37	-0.22	-0.21	-0.22	0.25	0.05	-	-0.13	-0.02	-0.21	-	-
Epidermal cell	-0.05	-0.17	0.25	0.26	0.25	0.07	0.03	0.11	0.06	-0.01	0.12	0.	-0.70
Harvest	0.24	0.31	0.04	-0.01	0.03	0.12	0.24	0.09	-0.25	0.20	-0.21	-	-0.01
Harvest	-0.25	-0.27	-0.04	0.02	-0.03	-0.06	0.10	0.05	0.35	0.04	0.38	0.	-0.14
Harvest	0.19	0.11	0.19	0.21	0.19	0.01	0.10	-	0.11	0.19	0.21	-	-0.10
Harvest split	-0.08	-0.15	0.05	0.08	0.05	-0.06	-	-	-0.04	-0.32	-0.10	0.	0.15
Harvest apex	-0.07	-0.02	-0.09	-0.14	-0.10	-0.02	0.06	0.04	0.07	0.02	0.03	0.	-0.06
7DAH	0.38	0.35	0.23	0.20	0.23	0.36	0.37	0.01	-0.26	0.15	-0.15	-	-0.09
7DAH broken	-0.03	0.14	-0.17	-0.11	-0.16	0.17	0.32	-	0.11	0.17	0.15	0.	-0.13
7DAH bruised	-0.34	-0.24	0.07	0.06	0.07	0.00	0.06	0.20	0.44	0.03	0.54	0.	-0.18
7DAH split	-0.29	-0.28	-0.14	-0.13	-0.14	-0.30	-	0.03	0.20	-0.21	0.11	0.	0.10
7DAH wilted	0.21	0.08	0.11	0.11	0.11	-0.08	-	0.20	-0.30	0.06	-0.33	-	0.02
Harvest	0.47	0.48	0.31	0.26	0.30	0.40	0.05	-	-0.52	0.16	-0.46	-	0.03
Harvest	-0.31	-0.29	-0.50	-0.43	-0.48	-0.17	0.24	-	0.60	-0.04	0.59	0.	-0.04
Harvest	-0.31	-0.24	-0.13	-0.13	-0.13	-0.36	0.15	0.10	0.34	-0.04	0.36	0.	-0.25
Harvest split	-0.23	-0.22	0.00	0.03	0.01	-0.19	0.05	-	0.10	-0.09	0.10	0.	0.26
Harvest apex	-0.32	-0.40	-0.18	-0.17	-0.18	-0.24	-	0.12	0.24	-0.23	0.13	0.	-0.10
7DAH	0.53	0.56	0.19	0.16	0.18	0.44	0.08	-	-0.44	0.17	-0.39	-	0.06
7DAH broken	-0.23	-0.33	-0.34	-0.31	-0.33	-0.13	0.50	0.11	0.42	-0.06	0.47	0.	-0.10
7DAH bruised	-0.17	-0.12	-0.07	-0.04	-0.06	-0.02	-	-	0.23	0.02	0.21	0.	-0.20
7DAH split	-0.41	-0.42	-0.07	-0.06	-0.06	-0.37	-	0.12	0.22	-0.17	0.15	0.	0.06
7DAH wilted	-0.23	-0.30	0.04	0.07	0.05	0.07	-	-	-0.05	-0.16	-0.06	0.	-0.01

	Epidermal cell area	Harvest undamaged washed	Harvest broken washed	Harvest bruised washed	Harvest split washed	Harvest apex tearing washed	7DAH undamaged washed	7DAH broken washed
	-							
Harvest	-0.19	-						
Harvest broken	0.31	-0.53	-					
Harvest bruised	0.21	-0.32	0.37	-				
Harvest split	-0.03	-0.86	0.17	0.20	-			
Harvest apex	-0.03	0.50	-0.63	-0.63	-0.46	-		
7DAH undamaged	0.09	0.65	-0.07	0.28	-0.62	-0.06	-	
7DAH broken	0.06	0.18	0.42	0.17	-0.29	-0.26	0.29	-
7DAH bruised	-0.04	-0.06	0.27	0.21	-0.11	-0.12	0.02	0.14
7DAH split washed	-0.08	-0.63	-0.03	-0.29	0.70	0.14	-0.90	-0.45
7DAH wilted	0.09	0.27	0.06	0.16	-0.30	-0.09	0.26	0.22
Harvest	-0.09	0.63	-0.44	0.08	-0.44	0.06	0.74	0.08
Harvest broken	0.02	-0.03	0.29	0.08	-0.18	0.05	-0.03	0.39
Harvest bruised	0.16	-0.48	0.44	0.23	0.30	-0.28	-0.26	0.19
Harvest split	-0.20	-0.38	0.25	-0.14	0.45	-0.19	-0.50	0.01
Harvest apex	0.20	-0.54	0.03	-0.26	0.45	0.25	-0.76	-0.49
7DAH undamaged	-0.15	0.62	-0.33	0.18	-0.46	-0.01	0.78	0.21
7DAH broken	-0.06	-0.01	0.11	0.16	-0.06	-0.01	0.04	0.19
7DAH bruised	0.48	-0.29	0.45	0.03	0.02	-0.07	-0.01	0.14
7DAH split	0.06	-0.52	0.10	-0.34	0.51	0.13	-0.83	-0.34
7DAH wilted	-0.13	-0.04	0.03	0.03	0.07	-0.12	0.13	0.04

	7DAH bruised washed	7DAH split washed	7DAH wilted washed	Harvest undamaged unwashed	Harvest broken unwashed	Harvest bruised unwashed	Harvest split unwashed	Harvest apex tearing unwashed
7DAH bruised	-							
7DAH split washed	-0.13	-						
7DAH wilted	-0.25	-0.48	-					
Harvest	-0.17	-0.68	0.21	-				
Harvest broken	0.25	-0.06	0.02	-0.37	-			
Harvest bruised	0.34	0.20	-0.24	-0.51	0.23	-		
Harvest split	-0.05	0.57	-0.24	-0.59	-0.02	0.28	-	
Harvest apex	-0.04	0.73	-0.16	-0.75	0.02	0.19	0.23	-
7DAH undamaged	-0.10	-0.73	0.20	0.93	-0.21	-0.50	-0.59	-0.78
7DAH broken	0.35	-0.01	-0.22	-0.33	0.68	0.31	0.07	0.09
7DAH bruised	0.02	-0.04	0.05	-0.11	0.19	0.41	-0.04	0.03
7DAH split	-0.09	0.83	-0.18	-0.79	-0.01	0.31	0.71	0.76
7DAH wilted	-0.09	-0.12	0.03	0.08	-0.03	-0.08	-0.10	0.01

7DAH undamaged	-				
7DAH broken	-0.24	-			
7DAH bruised	-0.18	-0.13	-		
7DAH split	-0.90	-0.01	0.08	-	
7DAH wilted	-0.04	0.14	-0.09	-0.12	-
	7DAH undamaged unwashed	**7DAH broken unwashed**	**7DAH bruised unwashed**	**7DAH split unwashed**	**7DAH wilted unwashed**

5.5 Spinach Experiment 4

Various biophysical characteristics were identified as being well correlated with the shelf life of either washed or unwashed leaves of babyleaf spinach (Table 12). These factors include: plant height, plant weight, plant leaf area, leaf thickness, SLA and leaf colour at harvest. These factors are strongly related to growth characteristics, illustrating the importance of crop maturity in the extension of shelf life.

Table 12: Summary of the factors that influence the shelf life of babyleaf spinach varieties.

Rank	Order [a]	Factor 1	Factor 2	Correlation
1	1	Plant height	Variety	0.83
2	2	Plant weight	Variety	0.71
3	2	Leaf area	Variety	0.67
4	2	Shelf life washed	Variety	-0.65
5	**2**	**Plant height**	**Shelf life washed**	**-0.64**
6	**2**	**Plant weight**	**Shelf life washed**	**-0.52**
7	**2**	**Leaf area**	**Shelf life washed**	**-0.52**
8	3	SLA	Variety	0.43
9	**3**	**Leaf thickness**	**Shelf life washed**	**0.40**
10	**3**	**SLA**	**Shelf life washed**	**-0.40**
11	**3**	**Leaf thickness**	**Shelf life unwashed**	**0.34**
12	**3**	**Leaf colour at harvest**	**Shelf life washed**	**0.33**
13	3	Leaf colour at harvest	Variety	-0.31
14	3	NO_3	Variety	0.30

[a] Order 1, correlation between ± 0.75 and 1.00; order 2, correlation between ± 0.50 and 0.75; order 3, correlation between ± 0.25 and 0.50.

Order 2 correlations for shelf life

The plant height of babyleaf spinach was shown to be strongly correlated with the shelf life of washed leaves. A higher plant height resulted in a reduced shelf life for washed leaves, while a lower plant height resulted in a longer shelf life (Figure 12). This response may be due to larger leaves being more susceptible to physical damage than smaller leaves.

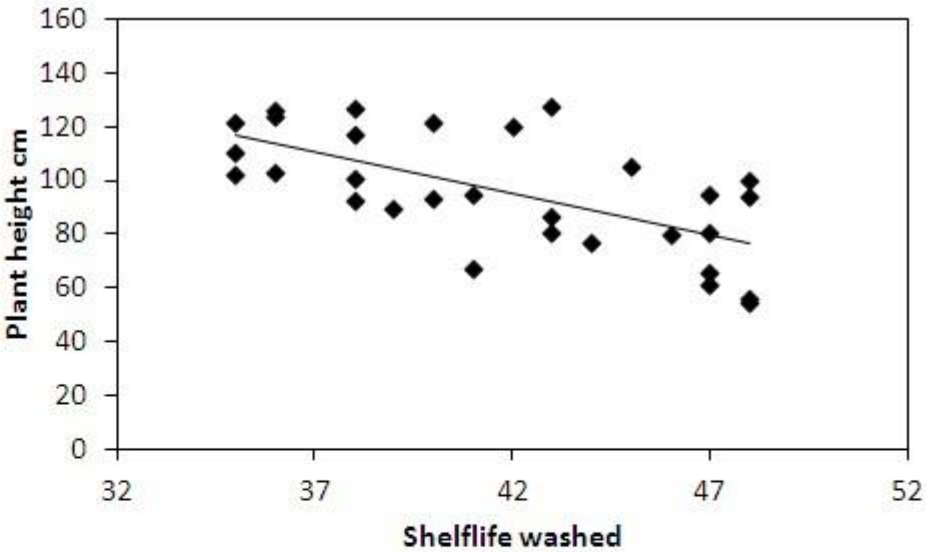


Figure 12: Relationship between plant height and shelf life of washed babyleaf spinach.

The fresh weight of plants was strongly correlated with the shelf life of washed leaves. A higher plant weight resulted in a reduced shelf life for washed leaves (Figure 13). This factor directly relates to plant maturity, with smaller lighter plants having superior shelf life when compared to larger heavier plants.

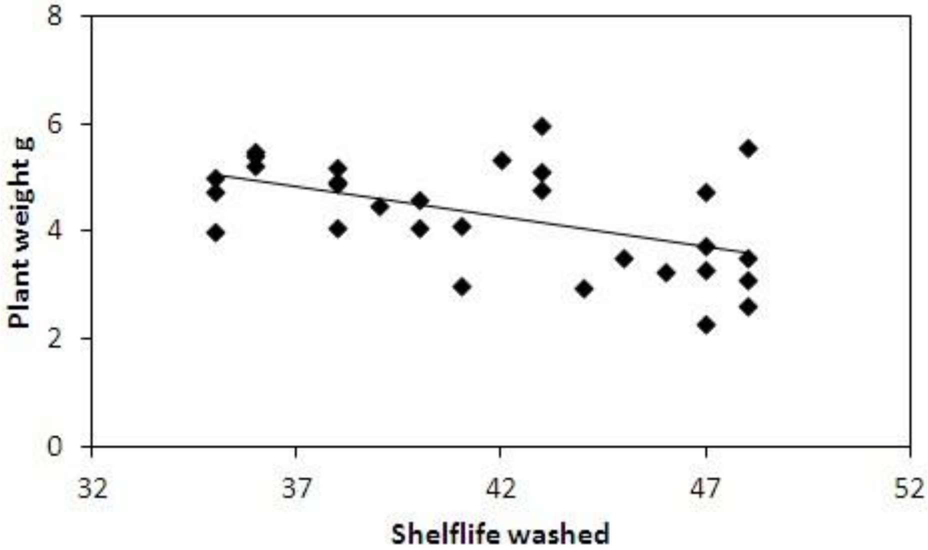


Figure 13: Relationship between plant weight and shelf life of washed babyleaf spinach.

The leaf area of individual plants was shown to be strongly correlated with the shelf life of washed leaves. A higher leaf area resulted in a reduced shelf life, while a lower leaf area resulted in a longer shelf life (Figure 14). This response is again influenced by the maturity of plants, with smaller leaves having longer shelf life than larger leaves.

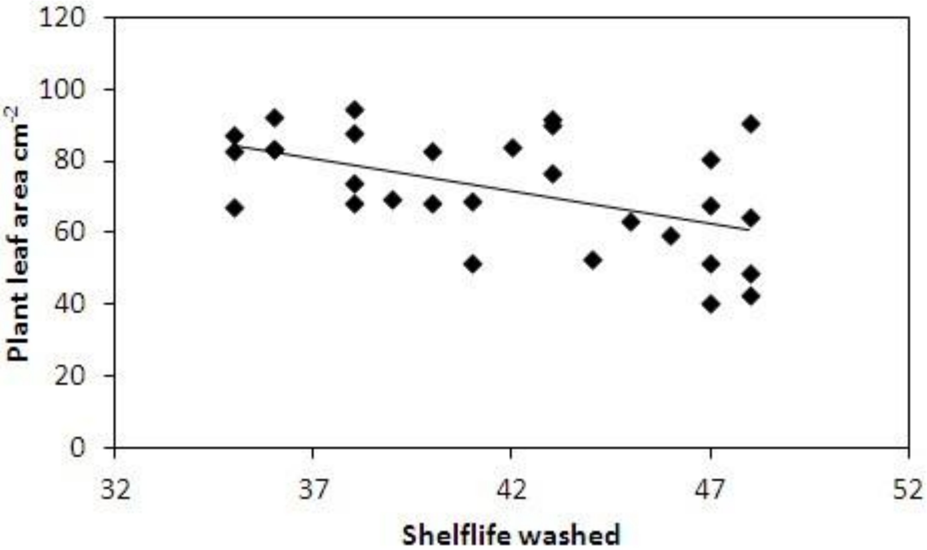


Figure 14: Relationship between plant leaf area and shelf life of washed babyleaf spinach.

Order 3 correlations for shelf life

Leaf thickness was correlated with the shelf life of washed leaves. A thicker leaf generally resulted in a longer shelf life (Figure 15). Thicker leaves may have been able to sustain a higher level of physical damage before shelf life was affected.

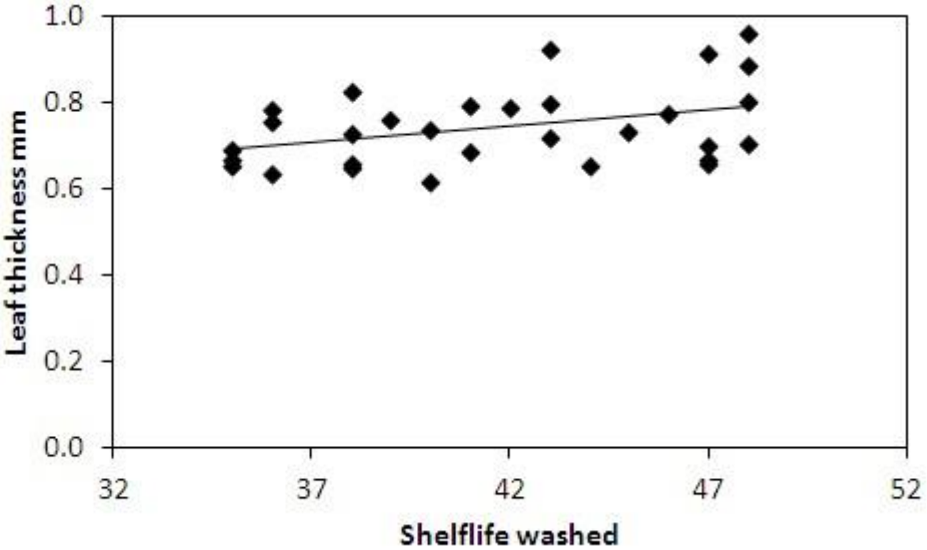


Figure 15: Relationship between leaf thickness and shelf life of washed babyleaf spinach.

The SLA of washed leaves was correlated with shelf life, with a lower SLA resulting in longer shelf life (Figure 16). This result was influenced by plant maturity and agrees with other negative correlations from this experiment.

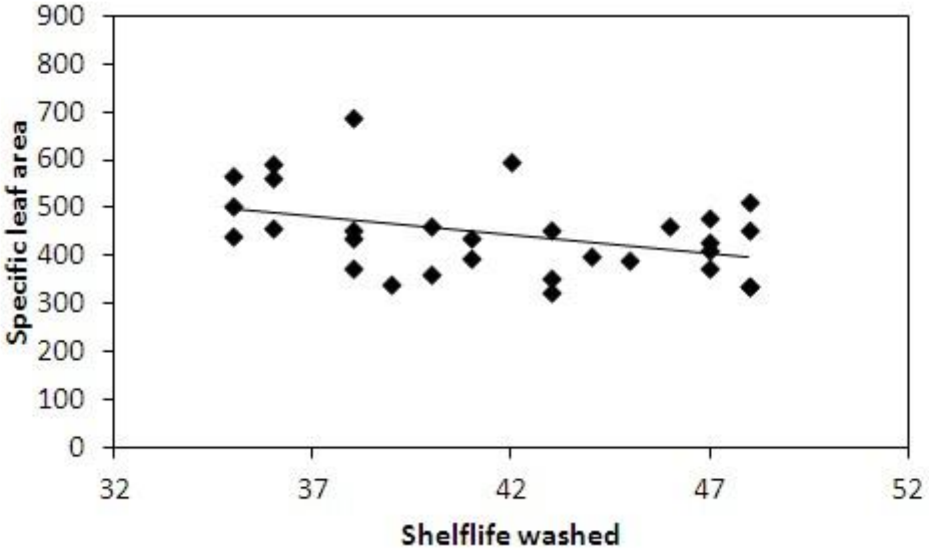


Figure 16: Relationship between specific leaf area and shelf life of washed babyleaf spinach.

Leaf thickness was positively correlated with the shelf life of unwashed leaves. A thicker leaf generally resulted in a longer shelf life for unwashed leaves (Figure 17). This relationship between leaf thickness and shelf life was also shown for washed leaves.

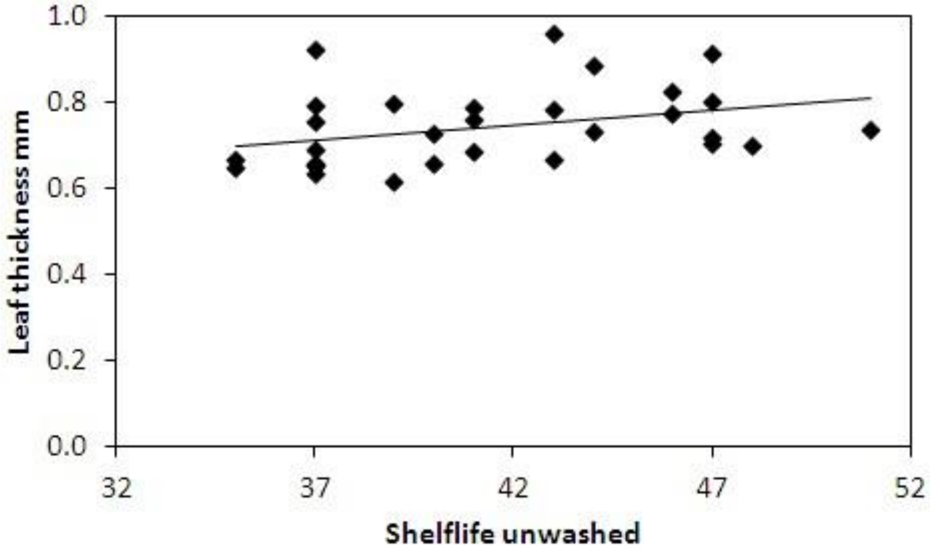


Figure 17: Relationship between leaf thickness and shelf life of unwashed babyleaf spinach.

The colour of leaves at harvest was correlated with the shelf life of washed leaves. The higher the leaf colour value at harvest the longer the shelf life (Figure 18). This response is related to the visual appearance of leaves, with higher colour values at harvest enabling leaves to remain greener for longer during storage.

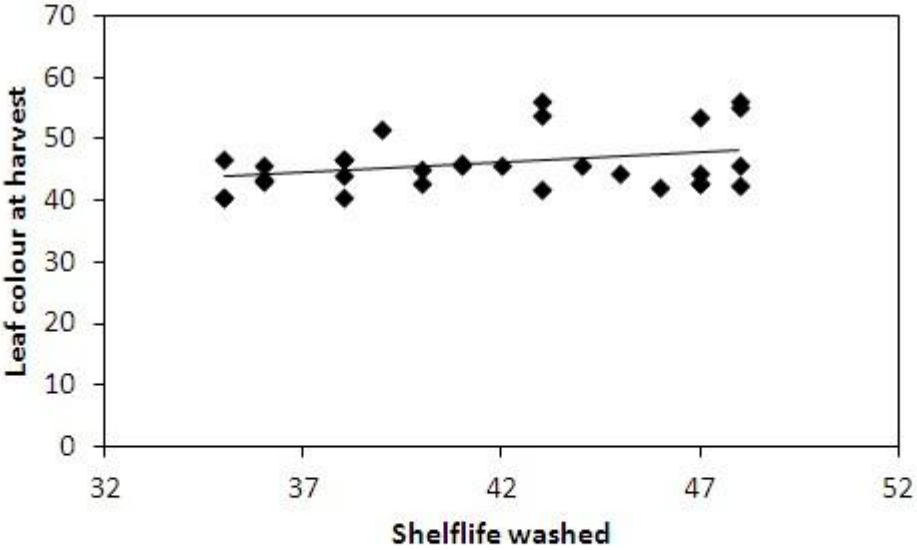


Figure 18: Relationship between leaf colour at harvest and shelf life of washed babyleaf spinach.

Table 13: Correlation matrix between variables measured and shelf life for experiment 4.

🟨 Order 1,
🟥 Order 2,
🟦 Order 3,

	Variety	Shelf life unwashed	Shelf life washed	Elasticity	Plant height	Respn 5°C	Respn 20°C	Leaf colour harvest	K	NO3	Plant wt	Leaf thickness	Leaf area
Variety	-												
Shelf life unwashed	-0.15	-											
Shelf life washed	-0.65	0.58	-										
Elasticity	0.12	-0.18	0.06	-									
Plant height	0.83	-0.06	-0.64	0.04	-								
Respiration 5°C	-0.02	0.01	-0.13	0.06	-0.06	-							
Respiration 20°C	-0.05	-0.14	0.23	0.17	-0.34	0.02	-						
Leaf colour at harvest	-0.31	-0.04	0.33	0.12	-0.65	0.38	0.49	-					
K	-0.17	0.05	0.03	-0.35	-0.07	0.29	-0.42	0.01	-				
NO3	0.30	0.22	-0.14	0.01	0.30	0.38	-0.16	0.03	0.3	-			
Plant weight	0.71	-0.19	-0.52	0.28	0.69	0.20	0.04	-0.12	-	0.2	-		
Leaf thickness	-0.08	0.34	0.40	0.23	-0.35	0.33	0.33	0.72	-	0.1	-0.03	-	
Leaf area	0.67	-0.08	-0.52	0.10	0.75	0.10	-0.06	-0.27	-	0.1	0.94	-0.16	-
SLA	0.43	-0.14	-0.40	0.20	0.64	-0.01	-0.30	-0.63	0.0	0.1	0.43	-0.31	0.4
Harvest undamaged	0.07	0.13	0.09	-0.21	-0.23	-0.13	0.26	0.27	-	-	-0.18	0.15	-
Harvest broken washed	0.01	-0.12	-0.16	0.16	0.25	0.24	-0.25	-0.19	0.0	0.4	0.24	-0.11	0.1
Harvest bruised	0.13	0.11	-0.10	0.00	0.24	-0.33	-0.32	-0.33	-	-	0.14	-0.12	0.1
Harvest split washed	-0.10	-0.38	-0.17	-0.36	-0.07	-0.16	0.16	0.07	-	-	-0.13	-0.23	-
Harvest apex tearing	-0.19	-0.01	0.18	0.37	0.05	0.08	-0.13	-0.24	0.0	0.2	0.03	-0.04	-
Harvest undamaged	0.15	0.17	-0.03	-0.19	0.07	0.13	0.05	0.00	-	-	0.12	-0.07	0.1
Harvest broken	-0.09	-0.08	0.13	0.22	-0.15	-0.16	0.03	0.12	0.3	0.1	-0.26	0.17	-
Harvest bruised	0.03	0.21	0.07	-0.17	0.20	0.10	-0.05	-0.13	0.0	0.2	0.39	-0.06	0.4
Harvest split unwashed	-0.29	-0.30	-0.26	-0.25	-0.09	0.27	-0.20	0.02	0.0	0.0	-0.01	-0.19	0.0
Harvest apex tearing	0.03	-0.11	-0.02	0.32	0.09	-0.27	-0.01	-0.18	-	0.1	0.02	0.01	-

	SLA	**Harvest undamaged**	**Harvest broken washed**	**Harvest bruised washed**	**Harvest split washed**	**Harvest apex tearing**	**Harvest undamaged unwashed**	**Harvest broken**
SLA	-							
Harvest	-0.36	-						
Harvest	0.25	-0.91	-					
Harvest	0.50	-0.03	-0.17	-				
Harvest	-0.19	-0.17	0.12	-0.13	-			
Harvest	0.32	-0.75	0.49	0.02	-0.13	-		
Harvest	-0.16	0.48	-0.50	-0.16	0.04	-0.25	-	
Harvest	0.05	-0.15	0.21	0.07	-0.15	0.02	-0.84	-
Harvest	0.19	-0.36	0.23	0.23	0.06	0.36	-0.10	-0.21
Harvest	0.02	-0.42	0.47	-0.22	0.47	0.11	-0.04	-0.26
Harvest	0.16	-0.34	0.29	0.29	-0.21	0.30	-0.68	0.37

	Harvest bruised unwashed	**Harvest split unwashed**	**Harvest apex tearing unwashed**
Harvest	-		
Harvest	0.03	-	
Harvest	0.11	-0.21	-

5.6 Rocket shelf life experiments

The total shelf life of European wild rocket was longest in summer, and then in winter and shortest in spring. It lasted longer when stored at 0°C compared to 5°C or 7°C. Across these conditions the amount of time taken for >50% of leaves to fail visual quality criteria ranged from 12 to 26 DAH (Figure 19).

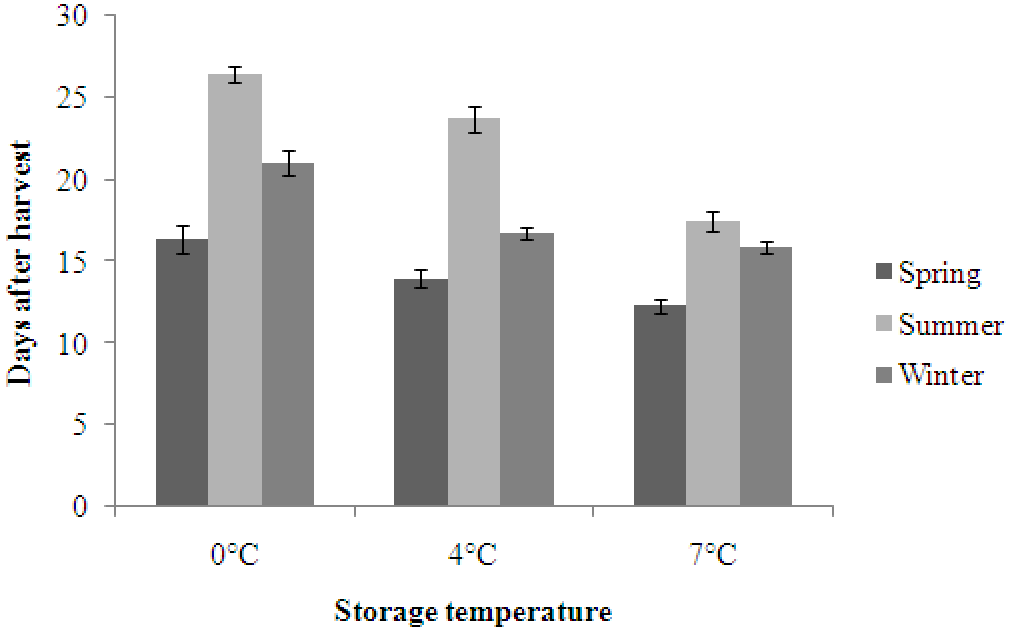


Figure 19: The shelf life of European wild rocket (*Diplotaxis tenuifolia* (L.) DC. (L.) DC.) leaves when grown during different seasonal conditions and stored at 0, 4, and 7°C. Values represent combined cultivar data for first and second harvests.

The shelf life of European wild rocket was significantly influenced by storage temperature across all harvesting events and seasons. European wild rocket lasted significantly longer when stored at 0°C compared to 4°C and 7°C, except for the second harvest spring crop (Table 14).

Table 14: The time taken for half of European wild rocket (*Diplotaxis tenuifolia* (L.) DC. (L.) DC.) leaves to fail visual quality criteria, when grown during different seasons of plant growth and harvest numbers.

Season	**Temperature °C**	**Shelf life (first harvest)**[*]	**Shelf life (second harvest)**[*]
Spring	0	20 a	13 a
	4	16 b	12 a
	7	14 c	11 b
	P value	*<0.001*	*<0.050*
	LSD	*1.61*	*1.55*
Summer	0	25 a	28 a
	4	21 b	26 b
	7	15 c	20 c
	P value	*<0.001*	*<0.001*
	LSD	*1.92*	*1.22*
Winter	0	19 a	23 a
	4	16 b	18 b
	7	15 b	17 b
	P value	*<0.001*	*<0.001*
	LSD	*1.28*	*1.95*

[*] Values rounded to the nearest day. Values with the same letter are not significant within seasonal harvesting events, *P*<0.05, least significant difference (LSD) 5% (n=12).

The total shelf life of arugula grown during winter showed the longest shelf life when stored at 0°C (22 DAH), 4°C (17 DAH), and 7°C (15 DAH), when compared to the other seasons of growth (Figure 21). The storage temperature influenced the shelf life with storage at 0°C resulting in the longest shelf life of leaves grown during different seasons (Figure 20).

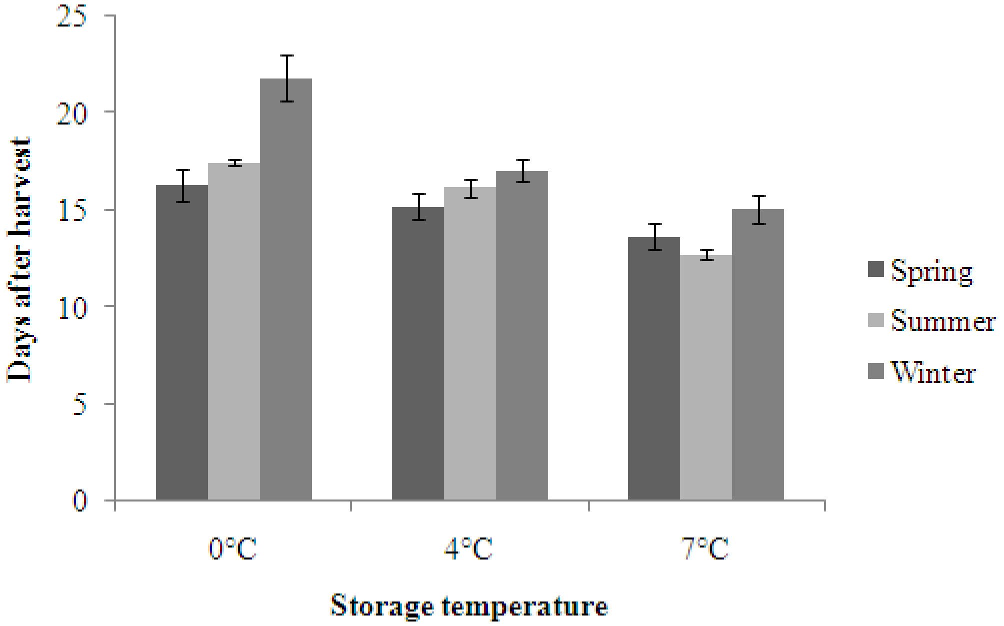


Figure 20: The shelf life of arugula (*Eruca sativa* Mill.) leaves when grown under different seasonal conditions and stored at 0°C, 4°C, and 7°C. Values represent combined cultivar data for first and second harvests.

The storage of leaves at 0°C resulted in the longest shelf life values of leaves grown during spring and winter conditions. However, during summer the shelf life was the same when stored at either 0°C or 4°C (Table 15). The shelf life of spring-harvested arugula was better for first harvest leaves compared with second cut leaves; the comparative shelf life within the same storage conditions varied by up to 8 DAH (Table 15).

Table 15: The time taken for half of arugula (*Eruca sativa* Mill.) leaves to fail visual quality criteria, when grown during different seasons of plant growth and harvest numbers.

Season	**Temperature °C**	**Shelf life (first) harvest)**[*]	**Shelf life (second) harvest)**[*]
Spring	0	20 a	13 a
	4	18 b	12 b
	7	16 c	11 c
	P value	*<0.001*	*<0.001*
	LSD	*0.95*	*0.33*
Summer	0	17 a	18 a
	4	16 a	16 a
	7	13 b	12 b
	P value	*<0.001*	*<0.001*
	LSD	*1.15*	*1.80*
Winter	0	18 a	26 a
	4	15 b	19 b
	7	13 c	18 b
	P value	*<0.001*	*<0.001*
	LSD	*1.26*	*2.00*

No significant differences were identified between the shelf life of European wild rocket cultivars when grown under different rates of nitrogen supply; as a result cultivar data was pooled. The shelf life of leaves grown under different rates of applied nitrogen and stored at 0°C ranged from 29 to 31 DAH, and were not significantly different (Table 16).

Table 16: The shelf life of European wild rocket (*Diplotaxis tenuifolia* (L.) DC. (L.) DC.) leaves when grown under different levels of nitrogen supply and stored at 0°C.

Nitrogen supply (N Kg ha^{-1})	**Shelf life (first harvest)**[*]
0	31 a
50	31 a
150	29 a
250	31 a
P value	*0.183*
LSD	-

[*] Values rounded to the nearest day. Values with the same letter are not significant across nitrogen application rates, $P<0.05$, least significant difference (LSD) 5% (n=12).

No significant differences were identified between the shelf life of arugula cultivars when grown under different rates of applied nitrogen for individual cultivars, therefore all cultivar data was pooled within rates of applied nitrogen. Shelf life of arugula leaves stored at 0°C was 28 DAH. Neither nitrogen supply nor cultivar had and significant effect on shelf life (Table 17).

Table 17: The shelf life of arugula (*Eruca sativa* Mill.) leaves when grown under different levels of nitrogen supply and stored at 0°C.

Nitrogen supply (N Kg ha^{-1})	**Shelf life (first harvest)**[*]
0	28 a
50	28 a
150	28 a
250	28 a
P value	*0.989*
LSD	-

[*] Values rounded to the nearest day. Values with the same letter are not significant across nitrogen application rates, *P*<0.05, least significant difference (LSD) 5% (n=12).

6 Discussion

6.1 Spinach

Experiment 1

The first experiment evaluated shelf life performance of 25 mechanically harvested spinach varieties in early autumn, at Camden, NSW. The crops were harvested mechanically and then assessed before and after commercial processing.

There was a good correlation between shelf life of the unwashed product and % weight loss after 7 days (-0.6), and % wilted leaves after 7 days (-0.59). Shelf life of the processed product was well correlated with % weight loss of the processed product (0.53), and shelf life of the unwashed product (0.49). Specific leaf area (SLA) and dry matter content were not well correlated with shelf life. SLA was well correlated with leaf dry matter (-0.87). There were very good correlations between weight loss and wilting, suggesting that processing does not significantly contribute to wilting or weight loss.

An interesting and consistent finding throughout the project has been that leaf bruising caused by harvesting, handling and processing completely disappears after 7 days in storage at 5^{o}C. Importantly, the results from the first trial suggested that processing per se did not have a major impact on shelf life.

The results suggest that bruising, damage and leaf dry matter content are not good predictors of shelf life, which contradicts current commercial thinking. To confirm this finding, the first experiment was repeated to confirm those conclusions, and to look for other predictors of shelf life.

Experiment 2

The second experiment was conducted on the same 25 varieties used for experiment 1, but this time grown during winter on the same property at Camden, NSW.

The crop was harvested mechanically and assessed initially unprocessed (without washing) and then processed (washed and spin dried). A second assessment was performed 7 days later after storage at 5°C in low-density perforated plastic bags. After the second assessment, samples were then stored at 5°C and shelf life assessed daily against the criteria of excessive desiccation and/or microbial breakdown.

The unprocessed babyleaf samples that best resisted damage were 10.98151, 09.78220, 10.98084, Squirrel, Crocodile and GB.25650. The most susceptible varieties were Road Runner, Swan and 09.78387. This damage was caused by harvesting and handling, not processing.

On the processed samples, the most resistant varieties were silver whale, 10.98151, Pelican, GB. 25650, Toucan, 10.98084, Emu, Pigeon and 9.78201. There was a small amount of leaf splitting after harvest, mainly on the varieties Pigeon, Marabu and 09.78387, and the amount of leaf splitting increased during processing.

Processing increased leaf visual damage but had little effect on the eventual shelf life and was also consistent with the findings from experiment 1. This was an unexpected but important result. It may have been due to relatively gentle processing equipment.

The bruising caused either by harvesting or processing almost completely disappeared after 7 days of storage at 5°C, which was consistent with findings from the first experiment.

There was an interesting trend in weight loss after seven days in storage, with half of the varieties losing from 7 to 8% in weight compared to the other half that lost only 2 to 3% of their weight. This suggests a varietal effect on resistance to desiccation.

On the unwashed samples there was a good correlation between shelf life and wilting at 7 days (0.51 and 0.54) and this was due to moisture loss through holes in the bags. There was also a good correlation between shelf life and bruising at 7 days (0.55), suggesting that bruising severe enough to still be there after 7 days is enough to cause a reduction in shelf life.

There was a correlation between respiration rate and bruising of the unwashed samples, which is consistent with the well-known response in plants to injury. There also is likely to be a corresponding increase in the rate of ethylene synthesis associated with this injury, and that may well be related to shelf life.

There was a negative correlation between respiration and bruising in the processed samples after 7 days, which is interesting and may be related to the slight improvement in shelf life after processing in some varieties. However, there was no clear relationship between leaf respiration rate and shelf life. This respiration work will be repeated in subsequent experiments and measured at a standard temperature, which may improve its predictive potential.

There were some weaker correlations between leaf thickness and shelf life, especially on the unprocessed samples, which suggests leaf thickness has a role in the determination of shelf life.

Experiment 3

Experiments 3 and 4 were carried out on a smaller subset of the varieties used in experiments 1 and 2. This subset was selected to provide the widest range in shelf life with a view to measuring a wider range of variables including sugars, starch, leaf anatomical and biophysical properties. Experiment 3 was grown in summer.

There was also a change in type of bags used in experiments 3 and 4; they did not have perforations but were ventilated daily as part of the shelf life assessment inspections. This change reduced shelf life losses due to desiccation, which is closer to commercial reality since commercial fresh cut bags are not perforated. Consequently, shelf life failures in experiments 3 and 4 were mainly microbial.

In general, leaves grown in experiment 3 and stored in unperforated bags at 5^{o}C had a shelf life range of 17 to 30 days.

Work by Zhang et al., (2007) suggested a range of quality trait loci (QTLs) that could be useful as tools in breeding programs to assist in the breeding and selection of long shelf-life lettuce. Variables such as leaf size, leaf weight, leaf chlorophyll content, leaf stomatal index, and epidermal cell number per leaf were strongly correlated with shelf life. Biophysical properties such as cell strength, elasticity and cell area (small cells) were also well correlated with shelf life, suggesting that the ideal idiotype lettuce should have small cells with strong cell walls. We used this study as the basis for investigating similar characteristics in spinach to assess their usefulness as predictive QTLs in spinach breeding work.

From the wide range of variables measured (see method section and appendix 3 for details), 6 variables were identified as having significant potential to be used as predictors of shelf life either in the field or as lab screening tests in a breeding program. Each of these characteristics is explained in the results and discussion section. The most promising indicators of shelf life were:

- Leaf colour (measured using a SPAD meter)
- Total chlorophyll (a+b) on a fresh-weight basis
- Specific leaf area
- Respiration rate at 20^{o}C
- Stomatal density
- Leaf thickness

While epidermal cell area was not itself well correlated with shelf life, it was highly correlated with stomatal density. This is an important finding and illustrates the connection between these two leaf morphological characteristics.

There was also a negative correlation between plant height and shelf life suggesting that smaller plants have a longer shelf life, probably by being able to better resist mechanical damage during processing.

Non-structural carbohydrates and shelf life were not well correlated, and while this was unexpected, it is a reasonable finding. Shelf life failures were mostly microbial and so exhaustion of stored carbohydrate reserves was unlikely to have come into play in this experiment. If microbial causes for failure of shelf life were effectively controlled, the exhaustion of carbohydrates may have become an important factor. There was a considerable range in non-structural carbohydrate levels between varieties, and this is valuable information for the future.

The following potential indicators were not well correlated with shelf life in this experiment:

- Sugars (glucose, fructose and sucrose)
- Starch and total non-structural carbohydrate
- Nitrate and potassium levels
- Epidermal cell area
- Elasticity

Experiment 4

The variables measured in experiment 4 (winter grown) correlated well with shelf life and mostly confirmed the results found in experiment 3 (summer grown). Perhaps the most striking difference, however, was that a maximum shelf of 50 days could be achieved at 5^{o}C compared to 29 days for summer-grown spinach. This is important because it indicates the potential shelf life achievable if growing conditions and postharvest storage are optimised.

In order of importance, for winter production the best negative correlations with shelf life were for:

- Plant height
- Plant weight
- Leaf area
- Specific leaf area

These results show that small plants and those with a low SLA (i.e. more dry weight per unit leaf area) have a longer shelf life than larger plants.

The following positive correlations with shelf life show that plants with thicker, darker green leaves last longer than plants with thin pale leaves.

- Leaf thickness
- Leaf colour

Measures of leaf elasticity and resistance to damage

The researchers had high expectations for the value of tests of physical strength, leaf elasticity, cell size and stomatal density would be good predictors of shelf life, however we found they are not as well correlated with shelf life in spinach as they are for lettuce. Simple field elasticity tests were not predictive of shelf life.

6.2 Rocket

Shelf life

The best shelf life of European wild rocket was observed in crops grown during summer. This is in contrast to the results achieved for spinach, where winter-grown has consistently had the longest shelf life. During summer conditions plants are also exposed to higher temperatures and longer day lengths resulting in greater radiant and thermal energy, and hence photosynthetic output. Therefore, the combination of lower levels of plant stress and longer warmer days may have resulted in an increase of stored reserves within the leaves which could be drawn upon during storage. Why this is not also true of spinach is an interesting question worthy of further investigation.

No significant differences in the shelf life between cultivars of European wild rocket were identified, indicating that commercially available cultivars of the species respond in a similar way during storage, and this may be due to selective breeding techniques that have resulted in the uniformed response of cultivars to abiotic factors.

Shelf life of European wild rocket was greater during winter conditions when compared to spring across a range of storage conditions. This has important commercial significance, particularly as growers are starting to produce European wild rocket during winter to satisfy retail market demands. The shelf life of leaves grown during winter conditions is therefore no worse than during seasons in which leaves are normally commercially grown (spring, summer, and autumn).

The temperature at which leaves were stored was shown to have a significant effect on the visual quality of leaves during storage, with storage at 0°C the most effective storage temperature for extending the shelf life of leaves. The extension of shelf life at this temperature has been reported in many leafy crops, and is due to the reduction of the respiration rate of leaves and the decrease of microbial spoilage (Farber et al., 2003; Koukounaras et al., 2007). The reduction in respiration rate prolongs the chlorosis of leaves and thereby maintains visual quality for longer. This relationship may also be influenced by the ascorbic acid (AA) concentration of leaves, which has been shown to prolong the visual quality of leaves during storage (Degl'Innocenti et al., 2007; Simões, 2004; Simões et al., 2010). At this temperature the shelf life of European wild rocket was extended by an average of 3 DAH when compared to storage at 4°C, and 6 DAH when compared to storage at 7°C. This represents a significant and commercially important extension of shelf life. That said, low temperatures remain difficult to achieve within the current commercial supply chain.

Arugula leaves had the longest shelf life when grown during winter at all storage temperatures, similar to the results for spinach, and there were no differences between cultivars. This was despite winter being outside of arugula's natural growth conditions, which are germination during late winter and spring followed by fast development with flowering occurring during summer (Pignone and Ngu, 1995). The longer shelf life achieved during winter conditions indicates that although this species does not, under natural conditions, grow during winter, cultivars of this species can produce commercially acceptable crops with a long shelf life.

This result may be explained by the fact that during winter arugula develops more slowly when compared to other seasons of growth, as differences in the amount of radiant and thermal energy slow the growth rate of plants. This relationship in combination with the fact that this species does not, under natural conditions, grow during winter may have resulted in leaves with higher levels of stored reserves, resulting from higher energy allocation into leaf structural components and less into reproductive structures (Meloche and Diggle, 2003; Palacio et al., 2007). This combination may have contributed to longer comparative shelf life for winter-grown arugula leaves.

The storage of leaves at lower temperatures was shown to significantly extend the visual quality of arugula leaves during storage, with the most effective temperature being 0°C. The storage of arugula at low temperatures is particularly important as it is a C_3 plant with a higher respiration rate during storage than European wild rocket, which is a C_3-C_4 intermediate plant (Byrd et al., 1992; Martínez-Sánchez et al., 2008a). When leaves of arugula were stored at 0°C the shelf life was extended by an average of 3 DAH when compared to storage at 4°C, and 5 DAH when compared to storage at 7°C. These results again reiterate the importance of storage temperature on the shelf life of high respiration rate, leafy vegetables leaves such as arugula.

The storage of rocket within the current supply chain is not optimal and is limited by open refrigerated display cabinets in stores where storage temperatures from 5°C to 10°C are common (Koukounaras et al., 2007; Wagstaff et al., 2010; Watada et al., 1996). Previous shelf life studies of European wild rocket have found this species to have a shelf life of approximately 14 DAH when stored at 4°C (Martínez-Sánchez et al., 2006a, b; Nielsen et al., 2008).

The results of this study indicate that European wild rocket stored at 7°C (which is closest to the actual storage temperature achieved within the current supply chain), concur with Martínez-Sánchez et al. (2006a, b), where a shelf life of 12 to 17 DAH was achieved. At this storage temperature leaves grown during different seasons recorded significantly different shelf life, with the longest value achieved during summer. Under the same conditions (7°C) arugula recorded a shelf life from 13 to 15 DAH. Therefore, when rocket is stored at temperatures achieved by current commercial practices the shelf life of arugula is more consistent across seasons of growth, and may be better suited to the current supply chain.

Although storage at 0°C remains difficult to maintain commercially, the results of this study indicate that rocket should be stored as close to 0°C as possible. The storage of leaves at lower temperatures represents a significant difference for retailers and prolongs the viability of produce until the point of sale, thereby reducing cost, waste, and the supply of poor quality produce leading to diminished consumer product loyalty.

Nitrogen

Nitrogen fertilizer is a significant input cost for farmers but it is essential for achieving the high yields growers need for financial viability (Steingröver et al., 1986; Rodríguez-Hidalgo et al., 2010). High rates of applied nitrogen are generally believed to decrease the shelf life of leafy crops such as rocket (Cantwell and Kasmire, 2002; Ishaque et al., 2009).

The results of this study show that applied nitrogen had no effect on the shelf life of either European wild rocket or arugula when stored at 0°C. This finding indicates that regardless of the amount of nitrogen applied (within the range tested) to rocket crops, the postharvest shelf life of leaves is not affected.

7 Conclusions

The main conclusions from this study were:

Spinach:

- The variety, growing season and growth rate (days to harvest) have a major effect on shelf life of babyleaf spinach.
- Small leaves and younger plants have longer shelf life than large leaves and taller, more mature plants.
- The amount of leaf apex tearing and splitting has a negative impact on shelf life.
- Moderate bruising caused by harvesting recovers after 7 days in storage at 5^{o}C but severe bruising does not recover and leads to a loss of shelf life.
- Minor damage, caused by processing such as might occur with good quality processing equipment, does not have a major effect on shelf life. Excessive damage during processing will cause loss of shelf life.
- Extremely long shelf life is possible, up to 50 days after harvesting when held at 5^{o}C, with minimal damage for winter-grown spinach using slow-growing varieties.
- Most leaf failures were due to microbial breakdown, not loss of chlorophyll or depletion of starch or sugar reserves in the harvested product.
- Useful indicators of potential shelf life in order of significance were:
    - Variety
    - Growth rate (season)
    - Specific leaf area (negative)
    - Leaf colour at harvest or after 7 days (positive)
    - Respiration rate at 20^{o}C or 5^{o}C (negative)
    - Total chlorophyll (b) on fresh weight basis (negative)
    - Plant height (negative)
    - Leaf thickness (positive)
    - Leaf area (total and individual) - negative
    - Stomatal density (positive)
    - Total leaf damage (negative)
    - Leaf apex tearing and splitting (negative)
- Characteristics not predictive of shelf life:
    - Leaf elasticity
    - Epidermal cell size
    - Starch, sucrose, fructose or glucose levels
    - Total non-structural carbohydrate levels
    - Chlorophyll on leaf area basis
    - Minor leaf bruising

Aspects such as leaf elasticity, strength and brittleness were not useful in our trials where the shelf life differences were mainly due to varieties. However, they are likely to be significant predictors of shelf life in some situations such as assessing the shelf life potential of very soft leaf from spring- and summer-grown crops.

Of these tests, the most useful for growers to predict the shelf life of a crop in the ground were:

- Leaf dry weight per unit leaf area
- Leaf thickness
- Leaf size
- Plant height
- Total leaf damage after harvesting

Rocket:

When stored at 7°C the shelf life of European wild rocket was 17 days for summer-grown rocket. Winter leaves had a slightly shorter shelf life of 16 days. In spring-grown European wild rocket the shelf life dropped to 12 days. Under the same storage conditions, arugula had a slightly longer shelf life that European wild rocket when grown during winter, with a shelf life of 15 days; followed by spring with a shelf life of 14 days, and summer 13 days.

The shelf life of both rocket species was significantly influenced by storage temperature, with storage at 0°C consistently resulting in a longer shelf life. The longest shelf life of European wild rocket was 26 days for summer grown leaves stored at 0°C; in comparison the longest shelf life of arugula which was 22 days for winter grown leaves stored at 0°C.

No significant differences were found in shelf life between cultivars of either rocket species, indicating that commercial cultivars of these species respond in a similar way to postharvest storage.

Nitrogen supply does not affect the shelf life of either rocket species, and to achieve maximum shelf life rocket species should be held as close as possible to 0°C.

8 Technology Transfer

The technology transfer has been focussed mainly on communicating results to the VC contributor; this will later be extended to include the wider community of growers and processors.

There have been regular communications between AHR and the VC contributor, both with the Australian team and with the breeding program directors and quality research staff in the Netherlands. In addition, individual trial reports have been sent to the VC contributor at the completion of each main trial, the results discussed and any necessary changes made to the methodology of subsequent trials. There has also been regular contact and discussions amongst members of the project team.

When the results are released, the project final report will be made available via the AUSVEG website, AHR website and HAL. An article summarising the main findings of the project will be made available for publishing in *Vegetables Australia* and *Good Fruit and Vegetables* at the end of the confidentiality period.

9 Recommendations

The most useful indicators of shelf life should be fine-tuned and adopted as predictive tests both for in-field use, and as breeding selection tools for shelf life, i.e., specific leaf area (SLA), respiration rate, leaf colour, respiration rate, total chlorophyll, leaf thickness, stomatal density, individual leaf area, plant height, leaf apex tearing or splitting and total leaf damage after harvest or processing.

Total leaf damage could be used as a predictor of shelf life performance after processing and this assessment could be used to predict the shelf life performance of varieties in response to processing equipment that causes moderate damage to crops.

More work should be done on determining the effect of high nitrogen availability and planting density on the shelf life of babyleaf spinach under different seasonal conditions. Two significant areas remain to be developed to further improve the reliability of babyleaf spinach and rocket shelf life produced in Australia. These are:

1. Quick, reliable tests that growers can use to predict what the shelf life of a crop that is in the ground will be once it is harvested, when it has been affected by adverse conditions during growth.

2. Actions that can be applied to adversely affected babyleaf crops before harvest to restore the shelf life to an acceptable level.

10 References

Boese, S. R. & Huner, N. P. A. (1990). Effect of Growth Temperature and Temperature Shifts on Spinach Leaf Morphology and Photosynthesis. Plant Physiology 94, 1830-1836.

Byrd, G.T., Sage, R.F., and Brown, H.R. (1992). A comparison of dark respiration between C3 and C4 plants. *Plant Physiology* 100: 191-198.

Cantwell, M., Rovelo, J., Nie, X. and Rubatzky, V. (1998). Specialty salad greens: postharvest physiology and shelf life in Proceedings of the 3rd International Symposium on Diversification of Vegetable Crops. Acta Horticulturae 467, 371 – 377.

Cantwell, M.I, and Suslow, T.V. (2002) Chapter 36. Postharvest handling systems: fresh-cut fruit and vegetables. Kader A.A. Editor. Postharvest Technology of Horticultural Crops, University of California Agriculture and Natural Resources Publication 3311. USA.

Cantwell, M.I., and Kasmire, R.F. (2002). Handling systems: Flower, leafy, and stem vegetables. In: Kander, A.A. (Eds.), *Postharvest technology of horticultural crops*. University of California Agriculture and Natural Resources Publication 3311. Pp. 423-432.

Cheng, L., Fengwang, M.A., and Ranwala, D. (2004). Nitrogen storage and its interaction with carbohydrates of young apple trees in response to nitrogen supply. *Tree Physiology* 24: 91-98.

Chory, J., Peto, C.A., Ashbaugh, M., Saganich, R., Pratt, L., and Ausubelb, F. (1989). Different roles for phytochrome in etiolated and green plants deduced from characterization of *Arabidopsis thaliana* mutants. *The Plant Cell* 1: 867-880.

Degl'Innocenti, E., Pardossi, A., Tognoni, F., and Guidi, L. (2007). Physiological basis of sensitivity to enzymatic browning in 'lettuce', 'escarole' and 'rocket salad' when stored as fresh-cut products. *Food Chemistry* 104: 209-215.

Farber, J. N., Harris, L. J., Parish, M. E., Beuchat, L. R., Suslow, T. V., and Gorney, J. R. (2003). Microbiological safety of controlled and modified atmosphere packaging of fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety* 2: 142-160.

Ishaque, K., Iqbal, M., Jilani, M.S., and Waseem, K. (2009). Effect of nitrogen levels on lettuce growth and yield. *Journal of Agricultural Resources* 47: 405-412.

Koukounaras, A., Siomos, A.S., and Sfakiotakis, E. (2007). Postharvest CO2 and ethylene production and quality of rocket (*Eruca sativa Mill.* Mill.) leaves as affected by leaf age and storage temperature. *Postharvest Biology and Technology* 46: 167-173.

MacKinney, G. (1941). Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry* 140: 315-322.

Martínez-Sánchez, A., Allende, A., Bennett, R.N., Ferreres, F., and Gil, M.I. (2006a). Microbial, nutritional and sensory quality of rocket leaves as affected by different sanitizers. *Postharvest Biology and Technology* 42: 86-97.

Martínez-Sánchez, A., Marin, A., Llorach, R., Ferreres, F., and Gil, M.I. (2006b). Controlled atmosphere preserves quality and phytonutrients in wild rocket (*Diplotaxis tenuifolia (L.) DC. (L.) DC.*). *Postharvest Biology and Technology* 40: 26-33.

Martínez-Sánchez, A., Allende, A., Cortes-Galera, Y., and Gil, M.I. (2008a). Respiration rate response of four babyleaf *Brassica* species to cutting at harvest and fresh-cut washing. *Postharvest Biology and Technology* 47: 382-388.

Meloche, C.G., and Diggle, P.K. (2003). The pattern of carbon allocation supporting growth of preformed shoot primordia in *Acomastylis rossii* (*Rosaceae*). *American Journal of Botany* 90: 1313-1320.

Nicola, S., Fontana, E., Torassa, C. & Hoeberechts, J. (2006). Fresh-cut produce: Postharvest critical issues in Proceedings of the 4th International Conference on Managing Quality in Chains - The Integrated View on Fruits and Vegetables Quality. Acta Horticulturae 712, 223-230.

Palacio, S., Maestro, M., and Montserrat-Marti, G. (2007). Seasonal dynamics of non-structural carbohydrates in two species of Mediterranean sub-shrubs with different leaf phenology. *Environmental and Experimental Botany* 59: 34-42.

Pignone, D., and Ngu, M.A. (1995). Collection and conservation of rocket genetic resources: the Italian contribution. In: Padulosi, S. (Eds.), *Rocket Genetic Resources Network*. Report of the first meeting, 13-15 November, 1994, Legnaro, (Padova), Italy. International Plant Genetic Resources Institute, Rome, Italy, pp. 35-57.

Pratap, A., and Gupta, S.K. (2009). Biology and Ecology of Wild Crucifers. In: Gupta, S.K. (Eds.), *Biology and Breeding of Crucifers*. CRC Press, Boca Raton, FL, pp. 37-67.

Ranwala, A.P., and Miller, W.B. (2009). Comparison of the dynamics of non-structural carbohydrate pools in cut tulip stems supplied with sucrose or trehalose. *Postharvest Biology and Technology* 52: 91-96.

Rodríguez-Hidalgo, S., Artés-Hernández, F., Gómez, P.A., Fernández, J.A., and Artés, F. (2010). Quality of fresh-cut baby spinach grown under a floating trays system as affected by nitrogen fertilisation and innovative packaging treatments. *Journal of the Science of Food and Agriculture* 90: 1089-1097.

Shelton, A.L. (2004). Variation in chemical defences of plants may improve the effectiveness of defence. *Evolutionary Ecology Research* 6: 709-726.

Simões, A.N., Puiatti, M., Salomão, L.C.C., Mosquim, P.R., Puschmann, R. (2010). Effect in the quality of intact and minimally processed leaves of collard greens stored at different temperatures. *Horticultura Brasileira* 28: 81-86.

Steingröver, E., Woldendorp, J., and Sijtsma, L. (1986). Nitrate accumulation and its relation to leaf elongation in spinach leaves. *Journal of Experimental Botany* 37: 1093-1102.

Swink, F., and Wilhelm, G. (1994). Plants of the Chicago region. 4th ed. Indianapolis: Indiana Academy of science.

Ueda, Y. Ikeda, H., Imahori,Y. (1998). Quality and storability of spinach grown on solution culture (NFT) in summer and winter. Journal of the Japanese Society of Horticultural Science 67, 446-452.

Wagstaff, C., Clarkson, G.J.J., Zhang, F., Rothwell, S.D., Fry, S.C., Taylor, G., and Dixon, M.S. (2010). Modification of cell wall properties in lettuce improves shelf life. *Journal of Experimental Botany* 61: 1239-1248.

Watada, A.E., Ko, N.P., and Minott, D.A. (1996). Factors affecting quality of fresh-cut horticultural products. *Postharvest Biology and Technology* 9: 115-125.

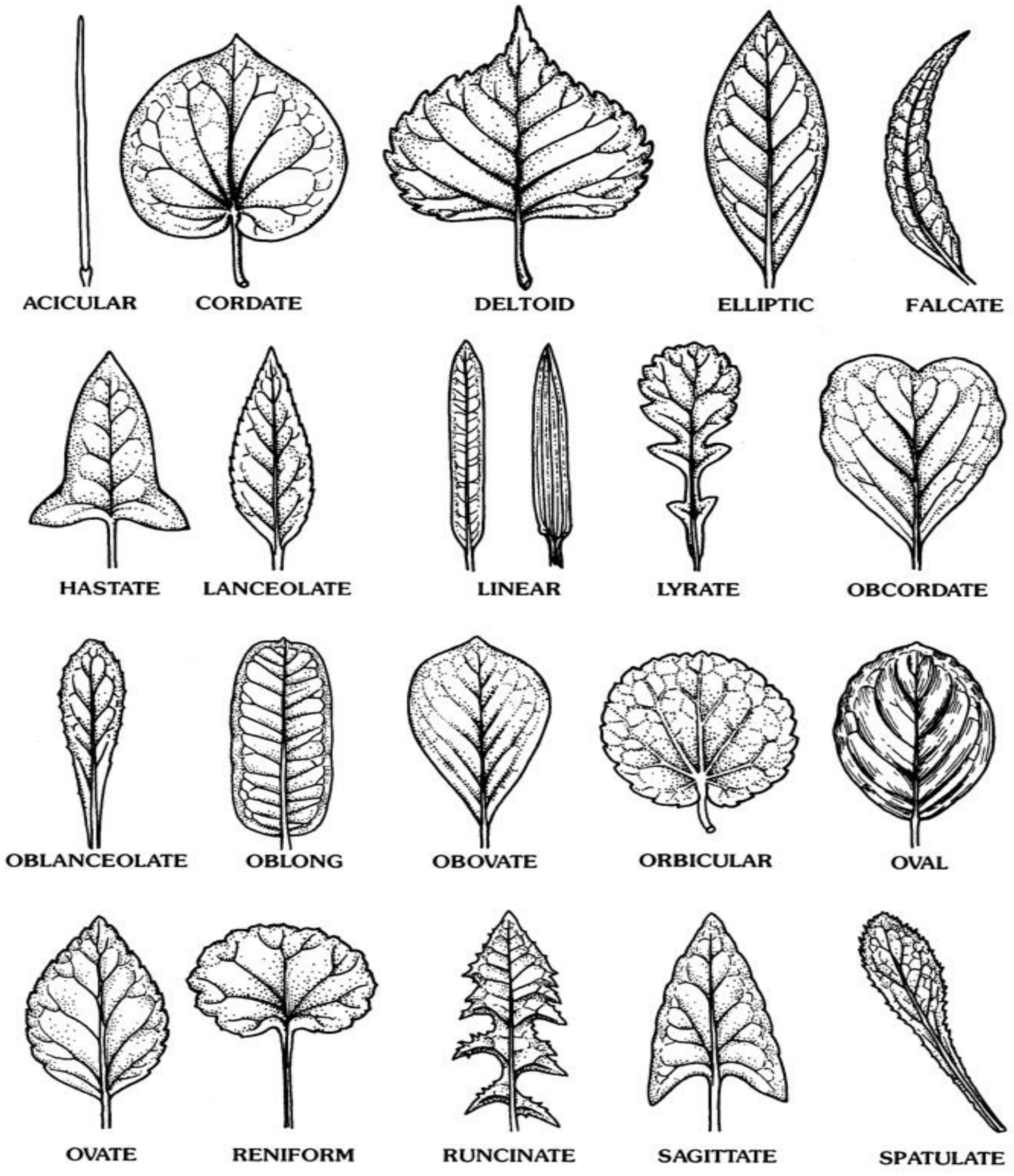
Yue, B., Cai, X., Vick, B., and Hu, J. (2009). Genetic characterization and molecular mapping of chlorophyll deficiency gene in sunflower (*Helianthus annuus*). *Journal of Plant Physiology* 166: 644-651.

Zhang, F. Z., Wagstaff, C., Rae, A. M., Sihota, A. K., Keevil, C. W., Rothwell, S. D., Clarkson, G. J. J., Michelmore, R. W., Truco, M. J., Dixon, M. S. & Taylor, G. (2007). QTLs for shelf life in lettuce co-locate with those for leaf biophysical properties but not with those for leaf developmental traits. Journal of Experimental Botany 58, 1433-1449.

11 Appendices

11.1 Appendix 1: Example of the leaf shape guide used to assess different varieties of babyleaf spinach.

PLATE 3. LEAF SHAPES



as published in Swink, F. and G. Wilhelm. 1994. *Plants of the Chicago region*. 4th ed. Indianapolis: Indiana Academy of Science.

11.2 Appendix 2: Example of leaf savoyness groupings.

11.3 Appendix 3: Example of leaf cupping groupings.

11.4 Appendix IV. Report on Visit to Rijk Zwaan Breeding station in The Netherlands

Gordon Rogers travelled to The Netherlands in October 2009 and visited Rijk Zwaan's research station at Fijnaart. The research station at Fijnaart employs about 200 people and the head office in De Lier employs about 600 people. Rijk Zwaan has operations in many countries including: France, Spain, Turkey, Germany, China, Australia (Daylesford and Gatton), Chile and Tanzania.

The company specialises in the breeding and production of high quality seed in crops such as lettuce, spinach, rocket, chard, leafy brassicas, tomatoes, cucumbers and many others.

Tour Rijk Zwaan Fijnaart and Inspection of the Research Babyleaf Washing and Processing Line

Rijk Zwaan has an experimental processing line, which was built specifically for testing the impact of processing (washing, drying and bagging) on babyleaf spinach, rocket, lettuce and other leafy salad vegetables. This pilot processing plant uses a commercial type of washer and spin dryer and can simulate the effects of normal commercial processing on babyleaf spinach.

The damage caused by processing and the susceptibility or resistance to this damage is of critical importance in the assessment of new babyleaf lines. It was concluded that in the current project we should include a processing step in our assessment of babyleaf salad vegetables.

The photos below show: the Rijk Zwaan washer and spin dryer; and an example of processed spinach that is being assessed for shelf life.

Photo 1 - Rijk Zwaan washer and spin dryer

Photo 2 – Processed spinach assessment for shelf life

Review of Babyleaf Breeding Material

The research farm and associated glasshouses are used in the breeding and assessment of new vegetable breeding lines. We inspected both the glasshouse facilities and the field, focussing on the new spinach breeding lines.

Photo 3 –New spinach lines in field trials, Rijk Zwaan breeding institute in Holland

Issues for babyleaf spinach:

- *Babyleaf types*: colour is important, generally the darker the better.
- *Leaf shape*: most markets favour elongated leaves, but UK still favours round leaves.
- *Splitting*: there is a common problem where the apex of the leaf splits – this is more of a problem in very cupped leaves. Slightly cupped leaves are OK.
- *Downy Mildew*: this is a major problem with spinach and a focus of the breeding program. There are now 11 strains identified worldwide and Australia currently has strains 1-7 only.
- *Bruising*: which occurs during harvesting, transport to the processing line or in processing itself. This is worse when the spinach is wet in the field.
- *Breaking of leaves*: this also occurs in harvesting and handling. The capacity of a leaf to resist breakage is likely to be an important factor.
- *Microbial contamination of leaves*: there is a very high level of surface microbial contamination on spinach leaves.

Spinach: The main spinach types and varieties assessed were:

- *Freezer types*: these are large leaf types and are mainly used to provide resistance to bolting.
- *Tuscan*: slower-growing type that gives better shelf life than fast-growing types. This confirms our findings from our previous HAL project on babyleaf spinach.
- *977*: very slow growing type – not yet released.
- *Roadrunner*: winter/spring/autumn variety; has reasonable shelf life because of the environment in which it is grown.
- *Squirrel*: splitting is the main problem because of very cupped leaves. Has good downy mildew resistance.
- *Sparrow*: flat pale leaves; an early variety.
- *Polarbear*: vigorous winter-type.
- *Beaver*: freezer variety with a pointed leaf, not favoured as a babyleaf type.
- *990*: new babyleaf type, leaves tend to stay in spec, long stems can damage other leaves and are a problem in processing.
- *Pelican*: highly susceptible to downy mildew.
- *Crocodile*: Savoy leaf type, not considered as slow-growing a type in Europe as it is in Australia. Withstands heat in Australia. Savoy leaf has performed well in washing studies. Leaves brittle in hot weather, resistant to sunburn, short stems.
- *703* and *Zebu*: semi-savoy types, tend to be brittle in hot weather, have long shelf life.
- *Red Kitten and Red Cardinal:* These red-veined types are not very popular at present but may have potential as an alternative to chard.
- Asian Types: *Elephant; Walabi; Dragon; Kangaroo*: These are mainly bunching types. The strength of the stem is important. They have more elasticity that the semi-savoy types.
- Asian Sumer Types: *131*: a slow-growing summer type.

The variety 703 might have about the right level of “savoyness”. Semi savoy types generally have good shelf life but tend to be susceptible to breakage (brittle). There should be a balance between these properties. Elasticity is also important and the semi savoy types need some more elasticity to resist damage, but the savoyness gives a nice amount of volume in the bag when packed.

Other Babyleaf Types Viewed

Salanova (Multileaf) Lettuce: An innovation by Rijk Zwaan seeds, this is a lettuce with a plant architecture that allows all the leaves to be cut by a single slice. The result is salad leaves that are ready to use individually, but a plant that is grown more like a conventional lettuce.

Meeting to Review Research Plan

This meeting was held with Rijk Zwaan staff at the research facility in Fijnaart on Monday 26th October 2009. Present at the meeting were: Pieter Egelmeers (head of plant breeding), Jan den Braber (spinach breeder), Frans Carre (rocket breeder), B. Kindhouts (pre-breeder), Manja Verhoef (postharvest quality assessment) and Bauke van Lenteren (Convenience manager).

The scope of the project was discussed and the detailed experimental plan revised as per the project proposal to ensure experiments and variables measured would deliver the outcomes expected. As a result, some changes to the experimental plan were requested. Basically, the changes were: the replacement of greenhouse studies with field studies because of the likely effect of the greenhouse on shelf life and the subsequent difficulty in accounting for this effect; focus on varietal and climate sources for variability (at the expense of nutrition and density studies); and a focus on spinach.

Itinerary of the visit

Date and Time	***Activity***
22-24/10/09	Travel to France via London and over night in Paris
25/10/09	Travel from Paris to Fijnaart via Amsterdam
26/10/09	
9.00	Tour Rijk Zwaan Fijnaart and inspection of the research babyleaf washing and processing line.
10.30	Inspect new babyleaf breeding material on the research station
12.30	Lunch
13.00 – 17.00	Discussion research project
19.00	Dinner
27/10/09	Visit surrounding farms in the region
28/10/09	Travel back to France
29-30/10/09	Travel back to Australia