### MINISTRY OF AGRICULTURE AND FISHERIES TE MANATU AHUWHENUA AHUMOANA



# THE PHYSIOLOGY OF POSTHARVEST DETERIORATION OF ASPARAGUS

REPORT TO THE N.Z. ASPARAGUS COUNCIL

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#### **SUMMARY**

We wish to understand the physiological processes accompanying the postharvest deterioration of asparagus so that ultimately we will be able to extend its storage life. Therefore, we have continued our studies on the metabolic and molecular changes that occur in spear tips after harvest.

#### Our main findings were:

- Postharvest changes in nitrogen metabolism (protein, amino acids, ammonia, glutamine synthetase activity) were similar over 48 h whether spears were stored in the light or the dark.
- Changes in gene expression can be detected within 6 h of harvesting asparagus.
- Shelf-life of spears decreases as the harvest season progresses.
- Nuclear magnetic resonance spectroscopy is a potentially useful tool for studying postharvest metabolism.



#### **INTRODUCTION**

In previous reports to Council we have identified many biochemical and physiological changes occurring in asparagus spears and tips after harvest. These include a rapid decline in respiration rate, loss of carbohydrate and protein, changes in amino acid composition, a build-up of ammonia and loss of cellular integrity.

We believe that these events hold the key to postharvest spear deterioration. Unravelling the biochemistry behind these events will provide the means by which the deteriorative process could be slowed. A slower rate of deterioration will extend storage life.

Most of our work is now done on spear tips cut from spears at intervals after harvest. Spear tips are the first part of the spear to show symptoms of postharvest deterioration. Hence, an understanding of the events that occur in spear tips after harvest should help us in our quest to control the postharvest deterioration of whole spears.

In this report we summarise our progress investigating the metabolic and molecular changes occurring in tips of asparagus spears stored for up to 48 h after harvest. We also describe the effect of harvest date on both shelf-life and changes in metabolism after harvest.



#### RESULTS

#### Respiratory Metabolism

Experiments were begun to find the cause of the rapid decline in respiration rate immediately after harvest and its effect on postharvest quality. Technical probelms hampered progress with these experiments, but we aim to overcome these problems next season..

We explored the use of nuclear magnetic resonance (NMR) spectroscopy as an additional method to monitor metabolic changes after harvest. Initial results were encouraging; we have verified the loss of carbohydrate and the accumulation of asparagine during postharvest deterioration. NMR spectroscopy has the potential to be used on living tissue (non-destructive analysis) and so offers exciting possibilities of following postharvest changes as they happen. We are collaborating with DSIR Chemistry, Petone, in this work.



#### Nitrogen Metabolism

Protein, composed of nitrogen-containing amino acids, is an important part of the spear tip, making up about a third of its dry weight. As the spear tip runs out of carbohydrate (sugars) after harvest, protein is broken down into amino acids which are subesequently used as respiratory substrates instead of sugars. The nitrogen-containing amino group remains as ammonia.

Ammonia is toxic to plant cells and it is normally detoxified by conversion into the amino acids glutamine and asparagine. Asparagine accumulates in spear tips after harvest but the tissue seems unable to cope with the release of amino groups, and ammonia accumulates after about 48 h.

Green plant tissue generally deteriorates faster when stored in the dark than in the light. Protein breakdown is often more rapid in darkness than in light, and in some tissues, asparagine accumulates only in the dark. We tested for differential effects of light and dark storage on nitrogen metabolism in asparagus spear tips. Table 1 shows that there was no difference between light and dark storage in the patterns of change for protein, ammonia, asparagine, glutamine, proline and glutamine synthetase. Glutamine synthetase is the enzyme that incorporates ammonia into glutamine.



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Table 1. Aspects of nitrogen metabolism in asparagus spear tips during storage at 20°C in continuous light (L) or continuous dark (D)

At harvest			Time after harvest (h)						
			3	6	9	12	18	24	48
Protein (mg/g dry wt)	<b>301</b>	L D	321 298	349 347	340 351	360 356	308 322	315 330	306 283
Ammonia	0.23	L	0.22	0.22	0.20	0.22	0.22	0.24	0.29
(mg/g dry wt)		D	0.22	0.20	0.22	0.19	0.23	0.22	0.25
Asparagine	6.3	L	3.7	4.3	5.1	5.0	7.2	12.8	25.1
(mg/g dry wt)		D	4.2	5.1	5.1	5.6	7.0	11.5	22.0
Glutamine	14.7	L	10.7	7.9	10.0	9.9	5.8	5.2	4.1
(mg/g dry wt)		D	10.4	10.1	7.8	7.4	6.5	5.3	3.7
Proline	4.1	L	1.9	1.2	1.2	0.9	0.8	0.7	1.8
(mg/g dry wt)		D	2.9	1.4	1.2	0.8	0.6	0.7	1.3
Glutamine synthetase (units/g dry wt)	130	L D	141 133	142 140	146 140	145 145	157 153	142 147	122 118



#### Time of Harvest

Although we know the sequence of physiological events occurring in spear tips after harvest, the timing of these events varies between experiments. For example, in last year's Report to Council we showed substantial ammonia accumulation between 24 - 48 h whereas the data in Table 1 show that ammonia is only beginning to rise after 48 h.

Asparagus is continually cropped from reserves built up during the previous summer. We thought that the time of the season at which the spears are harvested may influence our results because these reserves would be depleted by the end of the season and this may affect quality and storage life.

Figure 1 shows that shelf-life decreased from about 6 days to 3½ days over our harvest period. As well as having obvious commercial implications, this result may provide us with a marker for future work. If we can find out how early spears differ from late spears it should help us understand those factors that affect storage life. Unfortunately for our hypothesis, the decrease in shelf-life with later harvest dates appears unrelated either to the harvest levels of carbohydrate and protein, and their subsequent decline (Figures 2, 3) or to the rate of ammonia accumulation (Figure 4).



Figure 1. Effect of harvest date on shelf life

Spears were harvested on the dates indicated and held at 20°C and assessed

daily for shelf life.

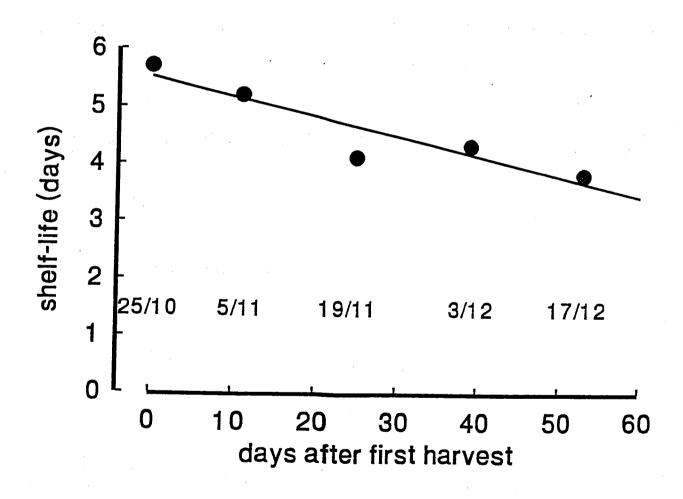


Figure 2. Effect of harvest date on soluble carbohydrate loss during shelf-life.

Inset shows harvest dates.

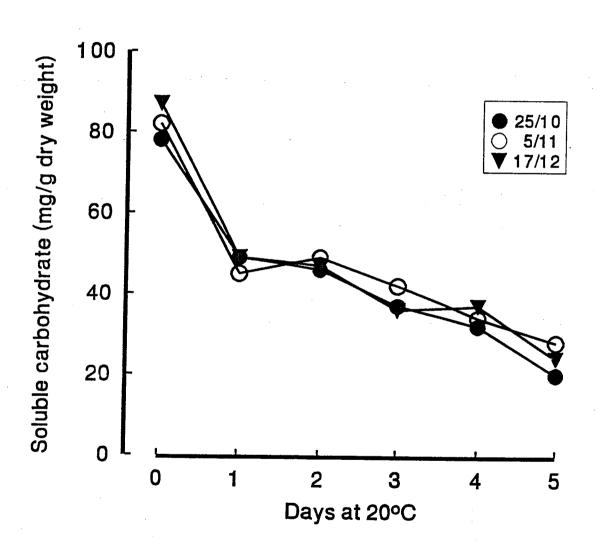


Figure 3. Effect of harvest date on protein loss during shelf-life.

Inset shows harvest dates.

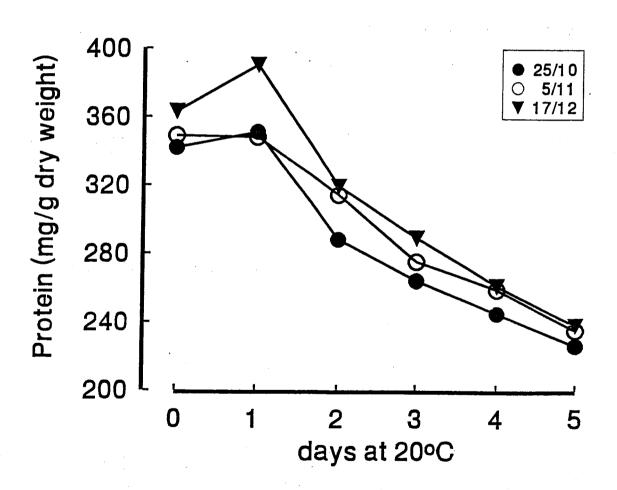
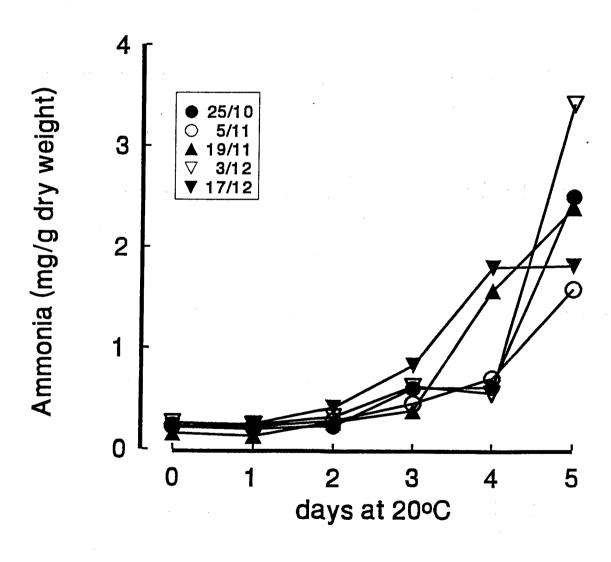


Figure 4. Effect of harvest date on ammonia accumulation during shelf-life.

Inset shows harvest dates.



#### Molecular Studies

All living cells have genes. Genes are composed of DNA (deoxyribonucleic acid) which contains the genetic information necessary to co-ordinate development in a single cell or in a whole organism. As required, the information encoded in the DNA is copied into messenger RNA (ribonucleic acid) and finally expressed as enzymes or proteins. Enzymes mediate all biochemical and physiological processes occurring in living tissue. The discipline studying the transfer of information from DNA  $\rightarrow$  RNA  $\rightarrow$  protein is called molecular biology.

Molecular studies of fruit ripening and cut flower deterioration are starting to contribute greatly to the understanding of these processes. For example, molecular studies of ripening tomatoes has helped unravel some of the regulatory mechanisms as well as giving opportunities for modifying the storage characteristics of the crop.

Asparagus is ideally suited for molecular studies. Tips of spears undergo rapid deterioration changes after harvest and we have characterised the associated biochemical and physiological changes. We have now found that changes in gene expression occur in tips after harvest. Some genes are turned on and some turned off, within 6 h of harvest - a very rapid response. We are now isolating and identifying these genes.

This gene expression work is being done in collaboration with the Plant Molecular Biology group at Levin and DSIR Crop Research at Lincoln. In the longer term, this work will allow testing of hypotheses on the control of deterioration, using plants that have been genetically modified in critical traits.



#### CONCLUSIONS

We have significantly extended our knowledge of the physiology of postharvest deterioration of asparagus.

- 1. Nitrogen metabolism in harvested spear tips is the same whether spears are stored in the light or the dark for 48 h.
- Initial molecular biology/gene expression studies have revealed that some genes are turned on and some genes are turned off within 6 h of harvest.
   We aim to identify those genes.
- 3. The shelf-life of spears decreases as the harvest season progresses. This has commercial as well as scientific implications.
- 4. NMR spectroscopy promises to be a useful technique for respiratory metabolism studies.

