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Quick test for the freshness of asparagus

A report prepared for the
New Zealand Asparagus Council

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CROP & FOOD RESEARCH
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1 EXECUTIVE SUMMARY

This report describes progress on a research project supported by both the NZ Asparagus Council and the Foundation for Research, Science and Technology via the Public Good Science Fund. Whilst the long term aim of our postharvest senescence research is to identify physiological, biochemical and molecular processes that cause deterioration in harvested perishable vegetables and flowers, we also aim to use the knowledge gained to develop simple tests that can monitor produce quality and predict postharvest life. Such tests could be used by industry either as quality assurance standards or as aids to maximise the marketing potential of produce after harvest.

Key points of the report are:

- Asparagine accumulation in asparagus is correlated with the postharvest temperature history, i.e. accumulated heat-units of the spears.

- Antibodies (from alfalfa) to asparagine synthetase react with protein extracts from asparagus and the intensity of the reaction increases with accumulated heat-units (degree-hours).

- The antibody reaction is more sensitive than asparagine as an indicator of spears' temperature history around the critical window of 500 degree-hours.

- The asparagine synthetase antibody reaction shows promise as the basis of a quick-test for the freshness of asparagus.

2 INTRODUCTION

Storage temperature is the most important factor in the loss of visual quality (freshness) of asparagus; the higher the temperature, the more rapid the loss of quality and the shorter the shelf-life. Indeed, the accumulated heat units (degree-hours; storage temperature x time) experienced by spears during postharvest handling is inversely related to shelf-life.

To maximise shelf-life, Crop & Food Research recommends that asparagus should not accumulate more than 500 degree-hours before it leaves New Zealand. However, direct calculation of degree-hours requires detailed postharvest time/temperature records. Usually this is not possible because the spears' postharvest temperature history is unavailable, so there is a place for a test which measures degree-hour exposure.

Previously, we have shown (and reported at the NZAC Research Seminar, 22 May 1996) that postharvest asparagine (a product of protein breakdown) accumulation in spear tips is highly correlated with degree-hours. Although spear tip asparagine levels and degree-hours were highly correlated ($R^2 = 0.878$), the relationship was not linear with a lag in asparagine levels resulting in little discrimination around 500 degree-hours. Therefore, a test based solely on asparagine levels in spear tips appeared not to be sensitive enough.

We have explored an alternative approach using a reaction system based on a protein involved in asparagine synthesis.

3 EXPERIMENTAL RESULTS

Freshly harvested 'Jersey Giant' commercial length asparagus spears were exposed to varying time/temperature conditions. Asparagine and soluble protein were extracted from freeze-dried and finely ground 3 cm spear tips.

3.1 Asparagine accumulation

Asparagine levels were determined by a colorimetric ninhydrin procedure. As shown in Figure1, we confirmed the strong relationship between asparagine levels and degree-hours. The graph also clearly shows that asparagine accumulation up to about 600 degree-hours is not significantly different from that at harvest (0 degree-hours).

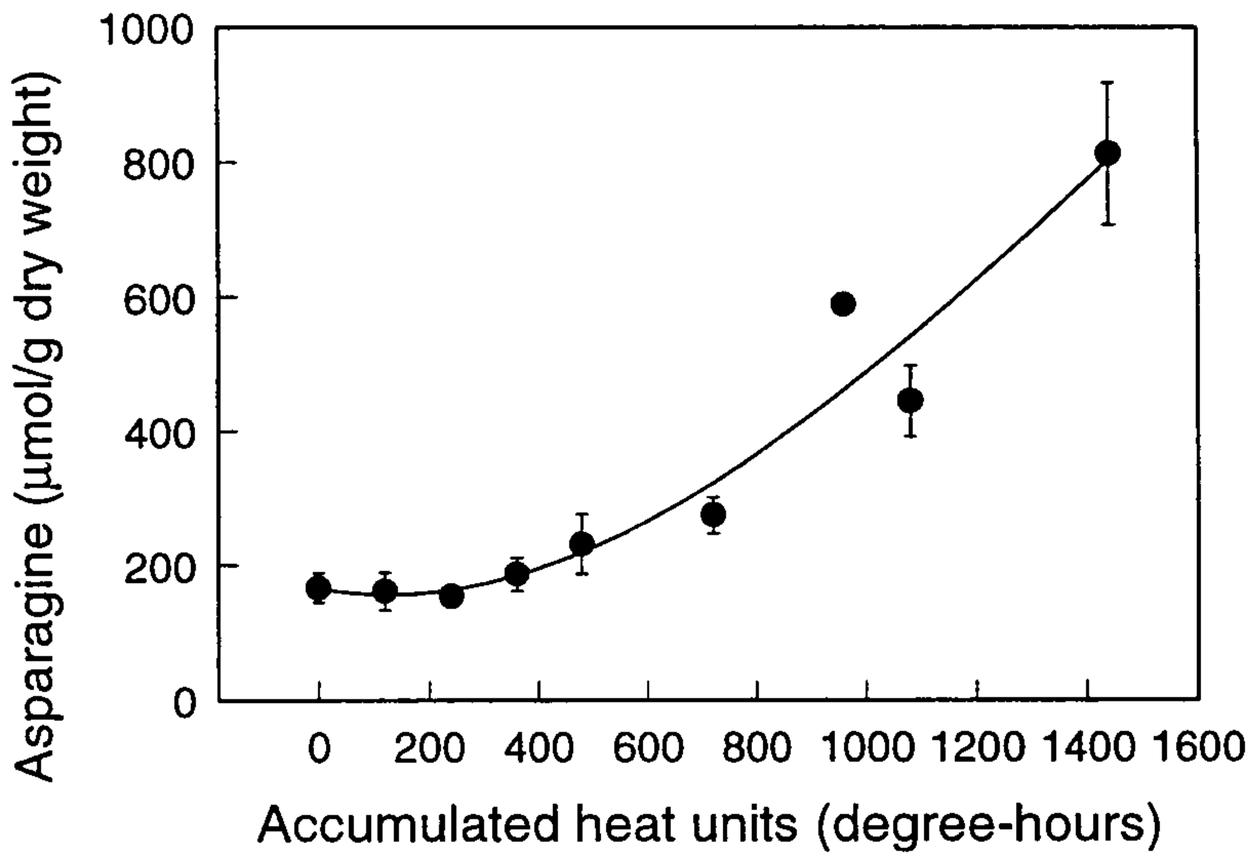

Figure1: Effect of heat accumulation on asparagine levels in spear tips

3.2 Asparagine synthetase antibody reaction

We know that the expression of messenger RNA for asparagine synthetase (AS), the enzyme that produces asparagine, is low in asparagus growing in the field but increases remarkably within a few hours of harvest. We have assumed that AS protein also follows this pattern, however, due to its unstable nature we have been unable to verify this by conventional assay. Although we cannot measure its enzymic activity, we can detect the AS protein by reaction with a suitable antibody. We have used antibodies, prepared against alfalfa AS, to see if there is a correlation in antibody response and accumulated heat units.

Briefly, the spear tip protein extracts were partially separated by electrophoresis, transferred to a membrane and reacted with the AS antibody and a mixture of chemicals to visualise the AS-antibody binding (Figure 2). Essentially, the darker the bands the more AS protein there is in the extract.

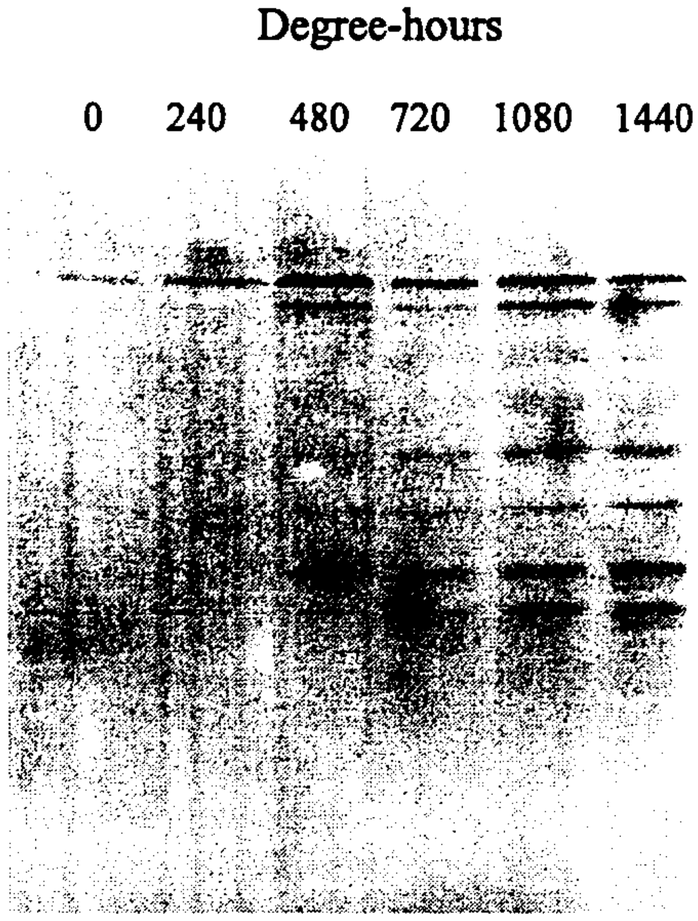

Figure 2: Reaction of asparagus tip protein extracts with AS antibodies

With the antibody reaction there is a strong response after 480 degree-hours, whereas it is much weaker at 0 and 240 degree-hours. Beyond 480 degree-hours, the response is variable. We believe this is due to the inherently unstable nature of the AS protein resulting in its natural degradation with time. AS degradation is also probably causing the appearance of the additional bands at higher degree-hours. These bands, which indicate smaller proteins (molecular weight of proteins decreases from top to bottom in Figure 2), represent AS degradation products that still have sufficient sites to recognise and bind the antibody.

The antibody reaction may be more predictable when proteins are not separated by electrophoresis (i.e., by using dot-blots), particularly beyond 480 degree-hours.

4 CONCLUSIONS

The asparagine synthetase antibody reaction system described in this report clearly has the potential to be developed into a quick-test that could identify spears that have been exposed to more than the recommended total of degree-hours. Further research is required, however, and would need to focus on:

- eliminating the protein extraction and electrophoresis steps, perhaps by using dot-blots;
- developing a spot-test that could be used in packhouses and would use juice expressed directly from spear tips;
- preparing asparagus AS antibodies to improve the test's specificity for asparagus.