ELSEVIER

PII: S0308-8146(97)00031-9

Food Chemistry, Vol. 61, No. 3, pp. 381–384, 1998

0308-8146/98 $19.00+0.00

Towards a freshness test for asparagus: spear tip asparagine content is strongly related to post-harvest accumulated heat-units

P. L. Hurst, G. Boulton & R. E. Lill

New Zealand Institute for Crop & Food Research Limited, Levin Research Centre, Private Bag 4005, Levin, New Zealand

(Received 20 September 1996; revised version received 9 December 1996; accepted 9 December 1996)

The shelf-life of asparagus (*Asparagus officinalis* L.) is strongly related to the post-harvest accumulated heat-units; the greater the number of accumulated heat-units (degree-h >0°C) experienced by the spears the lower the residual shelf-life. The potential of using asparagine and/or amino acid content as markers of freshness for asparagus spears was assessed by determining the relationship between the concentrations of these metabolites in the spear tips of two cultivars ('Limbras 10' and 'Jersey Giant') and the post-harvest age of the spears in terms of accumulated heat-units. There was a strong quadratic relationship between spear tip asparagine content and degree-h ($R^2 = 0.878$) that was independent of cultivar ($P = 0.16$). Free amino acid content was also correlated quadratically with degree-h ($R^2 = 0.788$ and 0.813 for 'Limbras 10' and 'Jersey Giant', respectively) but this was cultivar dependent ($P = 0.002$). Spear tip asparagine concentration has potential as a marker of freshness for asparagus but requires the development of an asparagine assay suitable for use in packhouses or by exporters.

INTRODUCTION

Fresh asparagus (*Asparagus officinalis* L.) is one of the most perishable of vegetables, having a marketable life of less than a week at ambient temperatures (Lipton, 1990). Storage temperature is the most important factor in the rate of loss of visual quality (freshness); the higher the temperature, the more rapid the loss of quality and the shorter the shelf-life (Lipton, 1990). Removal of field heat from the spears by hydrocooling before storage extends shelf-life (Lill, 1980). Two separate studies from our research centre have firmly established a strong negative relationship between shelf-life and the accumulated heat-units (degree-h > 0°C) experienced by the spears during post-harvest handling (King *et al.*, 1988*a*; Brash *et al.*, 1995).

Asparagus quality in both New Zealand's domestic and export markets is a key factor which determines success for the industry. Tight control of quality is rewarded by consistent demand, and by minimal wastage through minimising the rejection of consignments by exporters or importers. This control would be assisted substantially if packhouse operators and exporters had access to a quick test which measured the freshness, in terms of degree-h, of a particular line of asparagus so that its marketable life could be forecast. Crop & Food Research has recommended that the number of degree-h accumulated by asparagus should not exceed 500 prior to the commodity leaving New Zealand (Anon., 1993; Condie, 1993). Direct calculation of the accumulated degree-h requires knowledge of the time of harvest and the temperature history of the spears. Usually this information is not available, so there is a place for a test which measures the degree-h exposure.

We have observed that, during post-harvest storage of asparagus, the spear tip is usually the first part of the spear to show visual signs of deterioration (King *et al.*, 1993). Moreover, apical portions of spears lose sensory quality quicker than do basal portions (King *et al.*, 1988*b*), and tips undergo rapid and extensive metabolic change. In particular, there is a remarkable increase in the concentration of free amino acids, notably asparagine, in the tips (King *et al.*, 1990). The accumulation of asparagine is especially interesting in that it proceeds in an approximately linear manner (when measured daily) during 5 days of storage at 20°C (Hurst & Clark, 1993; Hurst *et al.*, 1993). Hence, it should be possible to use these compositional changes as indicators of spear freshness if the change occurs in a predictable fashion across a range of temperatures.

In this present study, the relationships between post-harvest accumulated heat-units (degree-h > 0°C) and

the content of free amino acids and asparagine in asparagus spear tips were investigated to determine whether they have potential for use as markers for freshness.

MATERIALS AND METHODS

Plant material

Commercial length (*ca* 200 mm) 'Limbras 10' and 'Jersey Giant' asparagus (*Asparagus officinalis* L.) spears were hand-harvested from respective field plantings at Levin Research Centre, New Zealand (site latitude 40.2°S, longitude 175.5°E) and a nearby commercial block between 0730 and 0800 h (NZ Daylight Time). Two replicate harvests, one week apart, were made. Those spears allotted to the 0 degree-h treatment were immediately packed in ice.

A single collection of 'Limbras 10' spears of varying length (110, 180 and 250 mm) was made in order to determine the effect of spear length on asparagine accumulation in spear tips. These spears were randomised into three replicates, and those replicates allotted to zero-time storage were kept on ice.

Storage regimes

Spears were transported to the laboratory, placed horizontally in plastic trays, covered with Glad® polyethylene cling-wrap and then placed in their allotted storage regimes within 1 h of harvest. For the accumulated heat-units experiment, spears were held at one of four temperatures (5, 10, 15, 20°C) for up to 48 h (12, 24, 48 h) to generate a range of post-harvest ages in terms of degree-h (temperature × time; 60–960 degree-h). Varying lengths spears were held at 20°C for up to 48 h (3, 6, 9, 12, 24, 48 h). Spears were kept in darkness except for brief periods of light required for removal of spears from storage. On removal from ice (zero-time and 0 degree-h samples) or storage, the spear tips (30 mm) were excised from spears and frozen at −15°C prior to being freeze-dried. For each temperature-time, or spear length-time storage regime, three tips were combined and considered as a single replicate for biochemical analysis. Freeze-dried tips were ground and stored desiccated at −15°C.

Biochemical analyses

Duplicate 10 mg samples of ground asparagus were extracted with warm 62.5% (v/v) methanol for 15 min (Haslemore & Roughan, 1976). The total free amino acid concentration in these extracts was estimated by the ninhydrin procedure of Magné and Larher (1992) with alanine as the standard. The asparagine (expressed as asparagine equivalents because of the interference by other amino acids in the method; Hurst *et al.*, 1995) concentration of the extracts from the degree-h spears was estimated by a modification (Hurst *et al.*, 1995) of the ninhydrin method of Sheng *et al.* (1993) with asparagine as the standard. Asparagine in the extracts from the varying length spears was quantified by high performance liquid chromatography (HPLC) as the *o*-phthaldialdehyde derivative as previously described (Hurst *et al.*, 1993). The HPLC method for asparagine was used for tips from varying length spears to enable direct comparison with our previous work using 18 cm spears (Hurst *et al.*, 1993, 1995).

Statistical and graphical analyses

Analysis of variance and general linear models procedures of the SAS package were used to test the effect of spear length and degree-h, respectively, on spear tip asparagine, and asparagine equivalents and amino acid contents. Curve fitting, regression analysis and graphic presentation employed the Axum® (TriMetrix Inc., 1992) package.

RESULTS

Although tips from shorter spears tended to accumulate more asparagine during storage at 20°C, this tendency just failed to reach statistical significance ($P = 0.06$). Accordingly, data pooled from the three spear lengths are presented in Fig. 1. Asparagine production during storage was linearly related to time ($R^2 = 0.947$, $P = 0.0002$) but a quadratic relationship gave a better fit ($R^2 = 0.997$, $P = 0.00001$). The quadratic relationship arises from the lag in asparagine accumulation apparent with frequent measurements during the first 24 h after harvest. Asparagine concentrations at

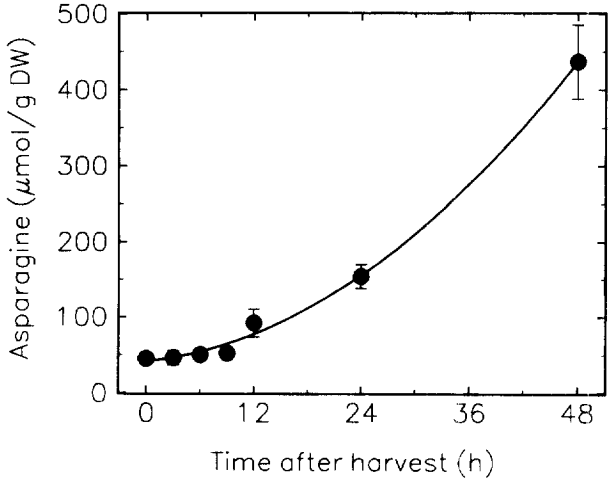

Fig. 1. Asparagine concentration in tips of 'Limbras 10' asparagus spears during storage at 20°C (spear lengths combined). Bars are SEM ($n = 3$) and where missing are contained within the symbols. The regression equation is $y = 0.145x^2 + 1.28x + 41.2$ ($R^2 = 0.997$, $n = 7$, $P = 0.00001$).

harvest, and after 24 and 48 h of storage agreed well with our earlier results (Hurst *et al.*, 1993, 1995).

The relationships between the total free amino acid content of spear tips, as measured by ninhydrin, and the degree-h exposure of spears of the two cultivars were also quadratic ($R^2 = 0.788$ and 0.813 for 'Limbras 10' and 'Jersey Giant' respectively, $P < 0.00001$; Fig. 2). Throughout storage, tips of 'Limbras 10' spears had significantly more ($P = 0.002$) free amino acid than did 'Jersey Giant' tips. However, this significant cultivar effect was lost ($P = 0.15$) when the more selective ninhydrin assay was used and asparagine equivalents estimated. Nevertheless, the quadratic relationship between the concentration of ninhydrin-positive compounds in spear tips, expressed as asparagine equivalents, and degree-hours of exposure remained ($R^2 = 0.878$, $P < 0.00001$; Fig. 3).

When the concentration of asparagine equivalents in spear tips from Fig. 3 was replotted against concentration of spear tip free amino acids from Fig. 2, a strong linear relationship was found ($R^2 = 0.830$, $P < 0.00001$; data not shown). This strong association is due partly to the interference of other amino acids in the ninhydrin asparagine assay and because the total free amino acid pool and asparagine concentration show the same pattern of post-harvest change, but also because asparagine is a high proportion of the total pool (King *et al.*, 1990; Hurst & Clark, 1993).

DISCUSSION

Although we have shown previously that the ninhydrin method of Sheng *et al.*, (1993) overestimates the asparagine content of asparagus spear tips (Hurst *et al.*, 1995), we have found the method useful here in measuring asparagine expressed as asparagine equivalents. This is because, despite the inaccuracy of Sheng *et al.*'s, (1993) method, it does give values that are highly correlated with those obtained by HPLC ($R^2 = 0.994$, $P = 0.003$; Hurst *et al.*, 1995). Furthermore, the concentration of asparagine equivalents in spear tips is positively correlated with days of storage at 20°C ($R^2 = 0.970$, $P < 0.001$; calculated from data in Hurst *et al.*, 1995). Thus, we believe that we are justified in using the ninhydrin asparagine method in this present work.

Several criteria have to be met if a particular biochemical change is to be used as the basis for developing a test for the post-harvest age and freshness of asparagus. These criteria include good definition in the critical window of post-harvest age (24–48 h after harvest), a change that correlates with the accumulated degree-h after harvest, and a change that is unaffected by commercial practice, such as cultivar and spear length at harvest.

We conclude that asparagine accumulation in the spear tip satisfies these criteria, and is thus worthy of further development. First, spear tip asparagine concentration increases significantly above baseline levels after harvest; second, the increase is strongly related to the post-harvest temperature history of the spears; and third, the increase appears to be independent of spear length and cultivar. This latter observation is important since a range of spear lengths from a variety of cultivars is likely to be gathered, depending on market requirements (Shewfelt & Mohr, 1960; Dean, 1993) and locality, respectively. Given that the relationship between shelf-life and accumulated heat-units is independent of cultivar (King *et al.*, 1988*a*), it is probable that the relationship between asparagine accumulation and

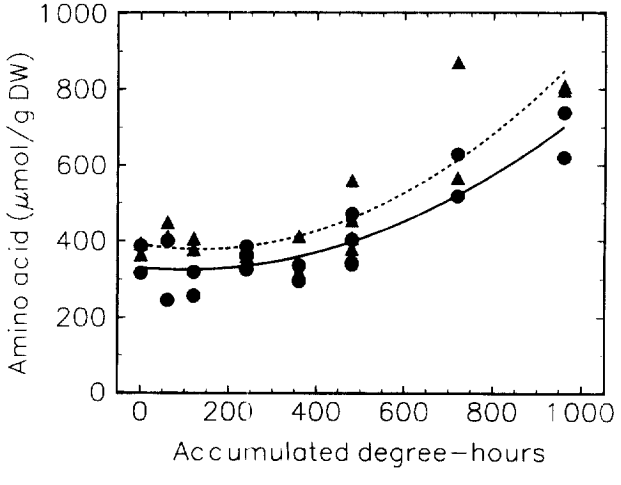

Fig. 2. Total free amino acid concentration in tips of 'Jersey Giant' (●) and 'Limbras 10' (▲) asparagus spears after varying degree-h exposure. The regression equation for 'Jersey Giant' (continuous line) is $y = 0.00051x^2 - 0.103x + 330$ ($R^2 = 0.813$, $n = 20$, $P < 0.00001$). The regression equation for 'Limbras 10' (dotted line) is $y = 0.00069x^2 - 0.187x + 391$ ($R^2 = 0.788$, $n = 20$, $P < 0.00001$).

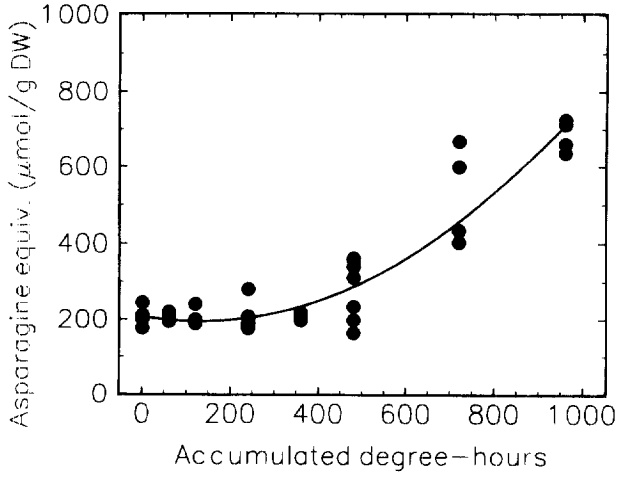

Fig. 3. Asparagine equivalents concentration in tips of 'Jersey Giant' and 'Limbras 10' asparagus spears after varying degree-h exposure (cultivars combined). The regression equation is $y = 0.00075x^2 - 0.193x + 207$ ($R^2 = 0.878$, $n = 40$, $P < 0.00001$).

accumulated heat-units, seen here with 'Limbras 10' and 'Jersey Giant', would also hold with other cultivars. However, a wider study is needed to verify this and to quantify the effects, if any, of other production factors.

Further work is needed to improve the sensitivity of the asparagine test if it is to be used to implement Crop & Food Research's recommendation. From the data in Fig. 3, an asparagine equivalents concentration of *ca* 400 μmol g^{-1} DW is sufficiently above baseline concentrations to be discriminatory. However, this equates with 650 degree-h, rather more than the 500 degree-h recommended by Crop & Food Research as the maximum which should be accumulated by the spears prior to leaving New Zealand (Anon., 1993; Condie, 1993). Further work is also required to devise an amenable analytical method for asparagine that is suitable for use by packhouses and exporters. Neither the time-consuming but precise HPLC method (Hurst *et al.*, 1993) nor the more rapid ninhydrin method, are suitable. A dry-chemistry reagent strip is a possibility, given that there is a specific colorimetric method for the detection of asparagine on paper chromatograms (Pasieka & Borowiecki, 1965). Such an approach may also improve test specificity and sensitivity.

Using endogenous biochemical changes as indicators of post-harvest freshness and quality for vegetable crops was advocated some years ago (Schwerdtfeger, 1979) and recently discussed again (King & O'Donoghue, 1995). However, few examples are proposed in the literature (Couture *et al.*, 1993; Yamane *et al.*, 1994). Our study adds to that number by showing that spear tip asparagine content is strongly related to the post-harvest accumulated heat-units, and thus asparagine production is a potential marker of freshness for asparagus.

ACKNOWLEDGEMENT

We thank Katie Jarmai-Graf for technical assistance.

REFERENCES

Anonymous (1993). Keeping asparagus cool. *Good Fruit & Vegetables*, **4**(7). 36.

Brash, D. W., Charles, C. M., Wright, S. & Bycroft, B. L. (1995). Shelf-life of stored asparagus is strongly related to post harvest respiratory activity. *Postharv. Biol. Technol.*, **5**, 77–81.

Condie, I. (1993). Keep it cool. *N. Z. Commercial Grower*, **49**(3), 27.

Couture, R., Cantwell, M. I., Ke, D. & Saltveit, M. R. (1993). Physiological attributes related to quality attributes and storage life of minimally processed lettuce. *HortSci.*, **28**, 723–725.

Dean, B. B. (1993). Yield and grade of asparagus harvested at three spear heights. *HortSci.*, **28**, 750.

Haslemore, R. M. & Roughan, P. G. (1976). Rapid chemical analysis of some plant constituents. *J. Sci. Food Agric.*, **27**, 1171–1178.

Hurst, P. L. & Clark, C. J. (1993). Postharvest changes in ammonium, amino acids and enzymes of amino acid metabolism in asparagus spear tips. *J. Sci. Food Agric.*, **63**, 465–471.

Hurst, P. L., King, G. A. & Borst, W. M. (1993). Post harvest inhibition of glutamine synthetase activity with phosphinothricin reduces the shelf-life of asparagus. *Postharv. Biol. Technol.*, **3**, 327–334.

Hurst, P. L., Sinclair, B. K. & Eason, J. R. (1995). Amino acids interfere with the ninhydrin assay for asparagine. *Food Chem.*, **53**, 467–469.

King, G. A., Henderson, K. G. & Lill, R. E. (1988*a*). Shelf-life of stored asparagus is strongly related to post harvest accumulated heat units. *Ann. Appl. Biol.*, **112**, 329–335.

King, G. A., Henderson, K. G., O'Donoghue, E. M., Martin, W. & Lill, R. E. (1988*b*). Flavour and metabolic changes in asparagus during storage. *Sci. Hortic.*, **36**, 183–190.

King, G. A., Hurst, P. L., Irving, D. E. & Lill, R. E. (1993). Recent advances in the post harvest physiology, storage and handling of green asparagus. *Postharv. News Inform.*, **4**, 85N–89N.

King, G. A. & O'Donoghue, E. O. (1995). Unravelling senescence: New opportunities for delaying the inevitable in harvested fruit and vegetables. *Trends Food Sci. Technol.*, **6**, 385–389.

King, G. A., Woollard, D. C., Irving, D. E. & Borst, W. M. (1990). Physiological changes in asparagus spear tips after harvest. *Physol. Plant.*, **80**, 393–400.

Lill, R. E. (1980). Storage of fresh asparagus. *N. Z. J. Exp. Agric.*, **8**, 163–167.

Lipton, W. J. (1990). Postharvest biology of fresh asparagus. *Hortic. Rev.*, **12**, 69–155.

Magné, C. & Larher, F. (1992). High sugar content of extracts interferes with colorimetric determination of amino acids and free proline. *Anal. Biochem.*, **200**, 115–118.

Pasieka, A. E. & Borowiecki, M. T. (1965). A specific method for the detection of asparagine on paper chromatograms. *Biochim. Biophys. Acta.*, **111**, 553–556.

Schwerdtfeger, E. (1979). Freshness as a part of vegetable quality. *Acta Hortic.*, **93**, 35–44.

Sheng, S., Kraft, J. J. & Schuster, S. M. (1993). A specific quantitative colorimetric assay for L-asparagine. *Anal. Biochem.*, **211**, 242–249.

Shewfelt, A. L. & Mohr, W. P. (1960). Effect of spear length, spear portion and harvest date on the composition and quality of asparagus. *Can. J. Plant Sci.*, **40**. 371–374.

TriMetrix Inc. (1992). *Axum*® Technical Graphics and Data Analysis, 2nd edition, TriMetrix Inc., Seattle, WA, 592 p.

Yamane, A., Yamane, A. & Shibamoto, T. (1994). Propanethial *S*-oxide content in scallions (*Allium fistulosum* L. variety Caespitosum) as a possible marker for freshness during cold storage. *J. Agric. Food Chem.*, **42**, 1010–1012.