Predictive Markers for the Optimisation of Thermal Treatments to Extend Shelf-Life in Minimally Processed Vegetables

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Introduction

Literature data indicate that mild thermal treatments can extend shelf life in a range of minimally processed vegetables by inhibiting browning reactions[1], inhibiting chlorophyll degradation[2], reducing surface lignification[3] and delaying microbial colonisation[4]. Despite these benefits, commercial uptake of the technology has been limited due to the need to optimise thermal treatment conditions which are dependent on cultivar, cut size and growing environment. As processor raw material is rapidly turned over, time consuming empirical optimisation of treatments is not appropriate within the commercial environment. Here we report the development and testing of predictive biomarkers for optimal thermal treatment based on the accumulation of small heat shock proteins or differential expression of genes encoding those proteins.

Results

Thermal treatments maintain quality of stored minimally processed vegetables

Thermal treatments improved visual appeal, texture and nutritional quality of stored products.

Figure 1 Impact of thermal treatments on yellowing of stored broccoli
Samples were harvested and treated in warmed water at the temperatures and times indicated then stored at room temperature for 9 days.

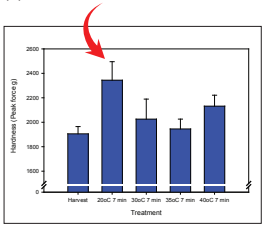

Figure 2 Impact of thermal treatments on hardening of carrot batons
Following preparation, carrot batons undergo a process of surface lignification (white blush) resulting in changes in texture. Samples were harvested and batons prepared and treated as indicated. The peak force required to drive a perspex blade thorough the batons at 5 mm min^{-1} was recorded using a QTS25 texture analyser (Brookfield Ltd., Essex) either immediately after harvest or following 7 days storage at room temeprature.

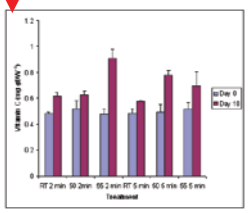

Figure 3 Impact of thermal treatments on vitamin C content of potato slices
Potatoes were harvested, peeled and cut into 5 mm slices. Immediately following treatment or after 10 days storage at room temperature, samples were extracted in 5% metaphosphoric acid containing 5 mM tris(2-carboxyethyl)phosphin e hydrochloride and ascorbic acid quantified by HPLC[5].

Optimal thermal treatment conditions are dependent on crop, cultivar and growing environment

In order for thermal treatments to have commercial application, it is desirable that the optimal treatment range is broad enough to be achieved at reasonable costs within a commercial environment. Optimal treatment ranges were therefore determined for a variety of crops by applying a matrix of temperatures and times. Following storage quality parameters were quantified to determine the impact of treatments on shelf life extension. Examples of optimal treatment ranges are provided in Fig. 4. Optimal treatments typically occupied a range encompassing 3 minutes by 5°C, well within the range controllable within a processing environment. However, optimal treatment conditions were variable according crop, cultivar and growing environment.

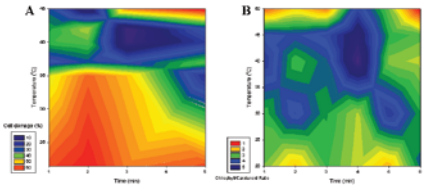

Figure 4 Definition of optimal thermal treatment regimes in two crops
Panel A – Impact of thermal treatments on cellular integrity in spinach leaves as determined by conductivity following 9 days storage at room temperature. Panel B – Impact of thermal treatments on yellowing in broccoli as determined by chlorophyll:carotenoid ratio following 9 days storage at room temperature.

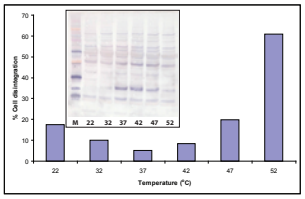

Figure 5 Impact of thermal treatment on cellular integrity and sHSP protein expression in spinach leaves
Leaves were treated for 2 minutes at the temperatures shown. One hour following treatment, proteins were extracted, separated on SDS-PAGE, blotted onto nitrocellulose membranes and sHSP protein expression was assessed using polyclonal sera raised in rabbit against *Arabidopsis* HSP17.6 (inset). Cellular disintegration was estimated using conductivity following 9 days storage.

Optimal thermal treatments correspond with expression of small heat shock proteins

Given the demonstrable benefits of thermal treatments in shelf-life extension and the feasibility of application at the commercial scale, the remaining barrier to uptake is the time consuming requirement to empirically define optimal conditions. To overcome this barrier, a series of biomarkers were investigated for use as predictive markers for shelf life extension following thermal treatment.
Of the biomarkers examined, the expression of small heat shock proteins most strongly correlated with optimal shelf life extension.

Figure 5 shows an example of small heat shock protein (sHSP) expression 1 h after thermal treatment of spinach leaves and the subsequent impact on cellular integrity following 9 days storage at room temperature.
While quantification of sHSP expression was shown to be a suitable predictive biomarker of optimal thermal treatment conditions, their use is limited to crops with sHSPs that cross react with available antibodies. Therefore the expression of sHSP encoding genes was tested as a potential biomarker. Figure 6 shows an example of correlation of sHSP gene expression with optimal treatment for browning reduction in potato.

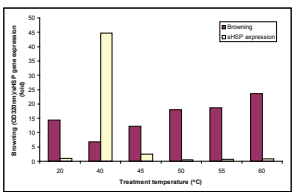

Figure 6 Impact of thermal treatment on browning and sHSP gene expression in potato chips
Freshly harvested potatoes were peeled and chipped prior to thermal treatment for 6 minutes at the temperature shown. One hour following treatment, RNA was extracted, cDNA generated and gene expression estimated using RT-PCR with primers designed to *Solanum peruvianum* HSP17.7 (Accession number AJ225047). Browning was determined by estinmation of OD 320nm of a methanol extract following 9 days storage at room temperature.

Conclusions and future objectives

- Optimal thermal treatments occupy a range that can be achieved within in a commercial environment.
- Predictive biomarkers have been identified to increase the efficiency of optimisation processes.
- We are now working in partnership with the processing industry to commercialise the use of thermal treatments for shelf-life extension.

Acknowledgements
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References
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