Measuring the Effects of Cold-Storage on the Chemical Composition of Potato Tubers using ^{1}H-NMR Metabolite Profiling

Catchpole, G. S. and Colquhoun, I. J.

Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, UK
E-mail: Gareth.catchpole@bbsrc.ac.uk

Introduction

The storage of potato tubers is a necessity if demand is to be met throughout the year as there is only one growing season in colder and temperate regions. Ideally, tubers could be stored at temperatures of around 4°C to prevent sprouting without the use of chemical inhibitors such as CIPC (isopropyl-N-chlorophenyl carbamate). However, storing tubers at such temperatures leads to significant sugar accumulation via the process known as “cool-induced sweetening”, which in turn leads to unacceptably dark-coloured fried potato products.

The current study gauges the effect of low- (2°C) and high- (20°C) temperature storage on the chemical composition of potato tubers from a cold-sweetening sensitive cultivar (cv. Bintje) using ^{1}H-NMR, uni- (ANOVA) and multi-variate (PCA) statistics. Despite previous investigations into the effect of storage temperatures on sugar levels, minor components have been mostly neglected. Therefore, the effect of storage temperature on other metabolites (amino acids, phenolic compounds, organic acids) is also determined.

Profiling by ^{1}H-NMR

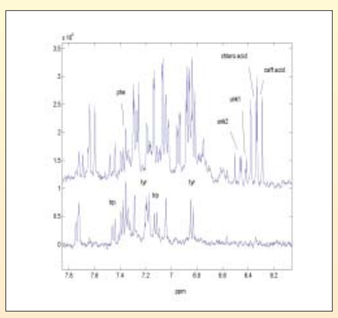

Fig 1a

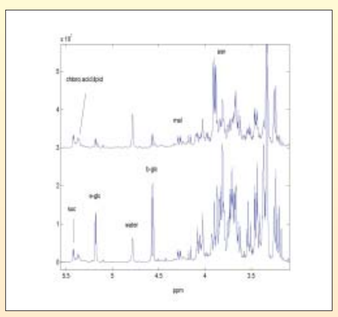

Fig 1b

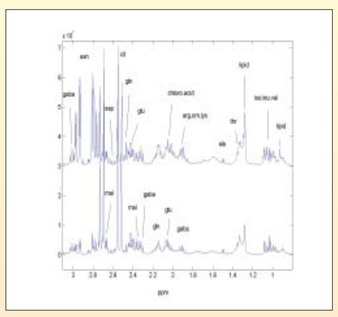

Fig 1c

Fig 1. Representative spectra from the flesh (lower) & peel (upper) of a potato tuber

Freeze-dried potato samples were extracted into 70% methanol-d / 30% 150mM buffer-d for 30mins at room temperature. Examples of typical 400MHz ^{1}H-NMR spectra resulting from potato peel and flesh can be seen in figure 1.

		Control	7	14	33
No storage	skin	9	n/a	n/a	n/a
	flesh	10	n/a	n/a	n/a
2°C	skin	n/a	10	10	9
	flesh	n/a	10	10	9
20°C	skin	n/a	10	10	10
	flesh	n/a	9	10	9

Table 1 – Showing the number of samples used in this study at days 7, 14 and 33

Analysis of Variance

Separate one-way ANOVAs were performed for each metabolite (using peak height) and the results displayed in the form of box plots. Significant differences were found between the means of groups stored at high and low temperature and are apparent with regards to glucose, sucrose, glutamic acid, aspartic acid and alanine in the flesh of tubers (figure 2). Significant differences relating to other constituents are evident, but these do not appear temperature dependent.

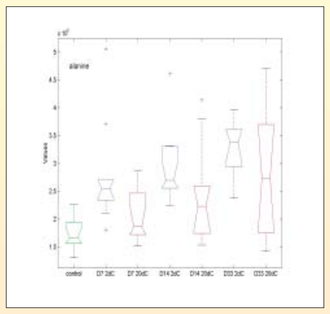

Fig 2a

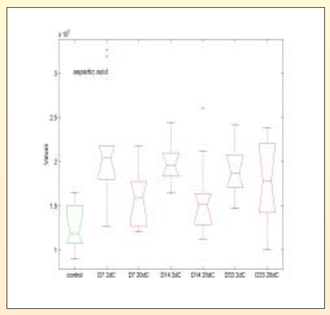

Fig 2b

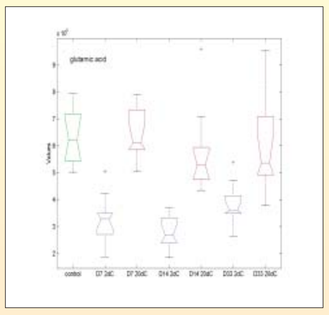

Fig 2c

Fig 2 Boxplots showing the trends displayed by the most significant amino acids in the flesh of tubers, alanine, aspartic acid and glutamic acid, in response to high and low temperature storage. Data is derived from NMR peak height. Green = controls; Blue = 2°C stored;Red = 20°C stored

The dominant amino acids in potatoes are asparagine and glutamine, but despite the level changes revealed by PCA loadings no statistically significant differences relating to the means of these compounds from each group are evident.

Similarly, storage temperature appears to have no influence on the content of the dominant phenolic compounds chlorogenic acid (5-*O*-caffeoylquinic acid) or caffeic acid, both of which are found almost exclusively in the peel of tubers. Significant differences are evident, but the level fluctuations appear to be storage duration dependent and are not a function of temperature (figure 3).

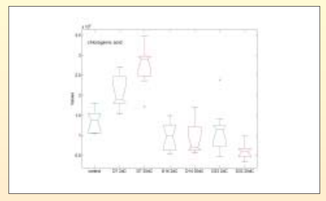

Fig 3a

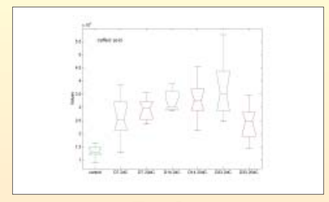

Fig 3b

Fig 3. Boxplots showing the trends displayed by the dominant phenolic compounds of the potato as found in the peel of tubers, chlorogenic acid and caffeic acid, in response to high and low temperature storage. Data is derived from NMR peak height. Green = controls; Blue = 2°C stored; Red = 20°C stored

Principal Components Analysis

The data set was analysed using PCA both including and excluding sugar resonances. It was apparent that the sugars were the dominant source of group discrimination due to the clear separation of groups at each time point when sugar signals were included in the analysis (data not shown).

Figure 4 shows that the groups from 2°C and 20°C can still be distinguished at each time point even when sugars were omitted, which indicates that minor components are also involved in the discrimination of groups. It is interesting to determine if the intensity of any of the distinguishing characters changes. Such changes demonstrate inconsistent behaviour in the level of the compounds concerned, resulting from different storage temperatures.

The important loadings from the PCA analyses at each time point are displayed in figure 5. The signals that notably alter intensity are labelled. The intensity of other signals also alters (e.g. asn, gln and tyr) but these are shown by ANOVA to be non-significant.

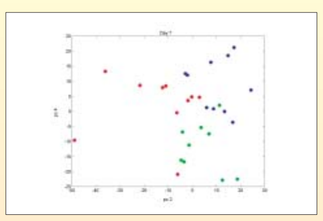

Fig 4a

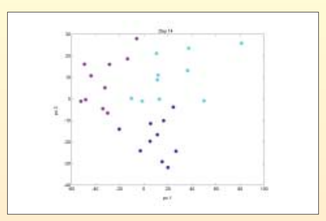

Fig 4b

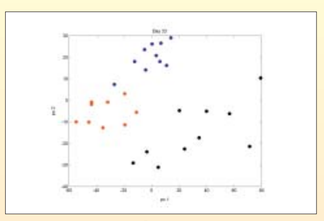

Fig 4c

Fig 4. PCA plots (correlation method) showing the separation attainable at each of the three time points. It is noticeable that the separation at day 7 is less well defined than the others. Blue = controls; Red = day 7 2°C; Green = day 7 20°C; Magenta = day 14 2°C; Cyan = day 14 20°C; Orange = day 33 2°C; Black = day 33 20°C

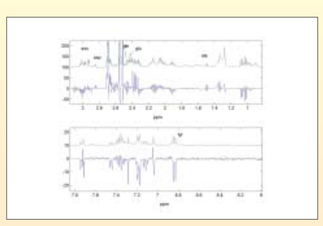

Fig 5a

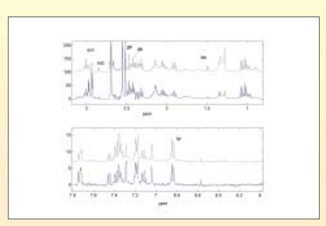

Fig 5b

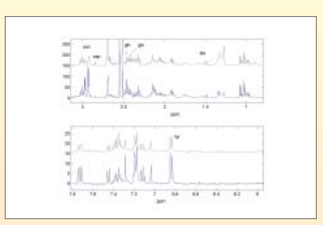

Fig 5c

Fig 5. Discriminating loadings (blue) and mean spectra (grey) from the PCA performed above. A = day 7; B = day 14; C = day 33

Conclusions

- Sugars are the compounds whose content is most obviously affected by differential storage temperatures
- 20°C storage – Sucrose remains at a low level, and glucose content decreases
  2°C storage – Sucrose level fluctuates and remains higher than in the controls, and glucose content increases (as does the concentration range)
- Of the amino acids alanine, aspartic acid and glutamic acid demonstrated clear effects of differential storage temperature:
  - Alanine – Mean content increases at both high and low temperature, but to a greater level at 2°C. Also, the concentration range greatly increases at 20°C which implies that only some tubers respond to high temperature storage
  - Aspartic acid – Content of high and low temperature stored tubers converges, but neither group shows significant change after the initial “shock response” of tubers when moved to low temperature storage, that being of a sudden content increase
  - Glutamic acid – Content decreases sharply on movement of tubers to low temperature. Remained at a lower level than that of the warm stored tubers which did not significantly change
- The remaining amino acids elicit differing responses, although some display similar trends e.g. glutamine and asparagine, isoleucine and valine, and tryptophan and tyrosine although storage temperature appears to have less influence than duration.
- The level of chlorogenic and caffeic acids fluctuates significantly, but this is also more likely to be a response to storage duration rather than temperature

Acknowledgements

The authors would like to thank BBSRC and Syngenta for funding this work as part of a BBSRC CASE studentship, and Dr Peter Howe of Syngenta for his assistance.