Chapter 19

APPLICATION OF METABOLITE AND FLAVOUR VOLATILE PROFILING TO STUDIES OF BIODIVERSITY IN SOLANUM SPECIES

Gary Dobson[1], Tom Shepherd[1], Rhoda Marshall[1], Susan R. Verrall[1], Sean Conner[1], D. Wynne Griffiths[1], James W. McNicol[2], Derek Stewart[1], and Howard V. Davies[1]
[1]*Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, UK;*
[2]*Biomathematics & Statistics Scotland, Dundee Unit, Dundee DD2 5DA, Scotland, UK*

Abstract: Volatile flavour compounds produced when potato tubers are boiled have been related to polar and non-polar metabolites present in raw tubers.

Key Words: boiling; cooking; flavour; gas chromatography; mass spectrometry; metabolite profiling; potato tuber; *Solanum tuberosum*; *S. phureja*; volatiles.

1 INTRODUCTION

Potato is a globally important foodstuff and source of nutrition and has been developed for agronomic traits such as yield and disease resistance. To meet changes in consumer demands, much effort is being put into improving other characteristics such as nutritional value and organoleptic properties. We are using a wide range of potato germplasm to explore phytochemical diversity. The diploid species *Solanum phureja*, developed from the Andean cultivated potato, has a yellower flesh than *S. tuberosum* due to higher carotenoid levels, has distinctive mouth-feel characteristics (high in smooth, and low in grainy and floury traits) and has more intense favourable flavour attributes (creamy, sour, earthy) than *S. tuberosum* (De, Maine et al., 2000). *S. phureja* genotypes are the subject of studies concerning the relationships between tuber metabolites, volatile flavour compounds and taste. The simultaneous analysis of polar metabolites from potato tubers has been carried out by gas chromatography-mass spectrometry (GC-MS) (Roessner

B.J. Nikolau and E. Syrkin Wurtele (eds.), Concepts in Plant Metabolomics, 259–268.

et al., 2000). In this study, GC-MS has been used to compare the polar and non-polar metabolites and volatile compounds from four *S. phureja* genotypes and four cultivars of *S. tuberosum.*

2 MATERIALS AND METHODS

2.1 Plant material

The plants used in this study were chosen on the basis of taste attributes determined by taste panels. The *S. tuberosum* cultivars Ailsa, Cara, Maris Piper and Pentland Dell represented "bland" cultivars, whilst the *S. phureja* genotypes (DB 257/28, DB 333/16, DB 337/37 and DB 358/23) were selected for their more "distinct" flavour (De, Maine et al., 2000).

Plants were field-grown using normal agronomic practices at a trial site (Mylnefield, Dundee, UK) in 2000. Tubers were harvested two weeks after foliage burn-down, kept at ambient temperature (ca. ~8–12°C) for 4 weeks to allow for skin set, then transferred to a 4°C store. At 4, 10 and 21 weeks post-harvest, *i.e.* 0, 6 and 17 weeks storage, two replicate samples of each genotype were taken from storage and used in the cooking experiments.

2.2 Isolation and analysis of tuber metabolites and volatile flavour compounds

For each replicate experiment, six average-sized tubers were chopped into eighths, two opposite eighths were taken for freeze-drying and the remainder were cooked by boiling. A further sub-sample was taken for freeze-drying after cooking, and the remaining tubers were sampled for volatile compounds. The freeze-dried samples were extracted and analysed for both polar and non-polar tuber metabolites by GC-MS. Volatile compounds produced during cooking were also analysed by GC-MS. Details of the procedures used for sample preparation, extraction, isolation and analysis are given in Chapter 15 (Shepherd et al., 2007).

3 RESULTS AND DISCUSSION

Comparisons were made between the four *S. phureja* genotypes and four cultivars of *S. tuberosum* on the basis of their polar and non-polar metabolite contents and compositions of volatile flavour compounds. The effect of low temperature storage was also studied.

3.1 Polar metabolites

The relative concentrations of 142 polar metabolites, including amino acids, sugars and organic acids were measured in each potato tuber sample. Data from replicate analyses of the four cultivars of *S. tuberosum* and four genotypes of *S. phureja* (raw and cooked) at all three storage dates were analysed by PCA and the two species were clearly separated (Figure 19-1A).

An examination of the specific metabolites responsible for the separation revealed that some amino acids (β-alanine, γ-amino butyric acid and proline) were elevated in *S. tuberosum,* whereas some aromatic (tyrosine and phenylalanine) and branched (*br*-) amino acids (leucine and isoleucine) were elevated in *S. phureja* (Figure 19-2), and the levels of valine, methionine and

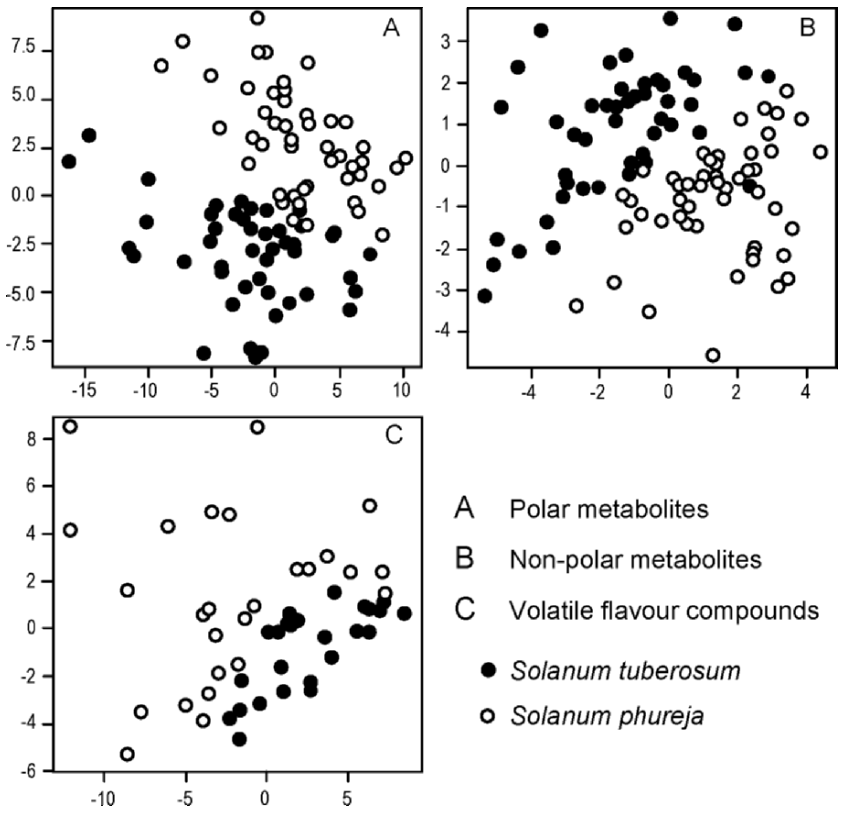

Figure 19-1. PCA plots showing separation of *Solanum tuberosum* and *S. phureja* in terms of (A) polar and (B) non-polar metabolites in cooked and non-cooked tubers and (C) volatiles from cooked tubers. Vertical axis: score (3); horizontal axis: score (1) for (A, C), score (2) for (B).

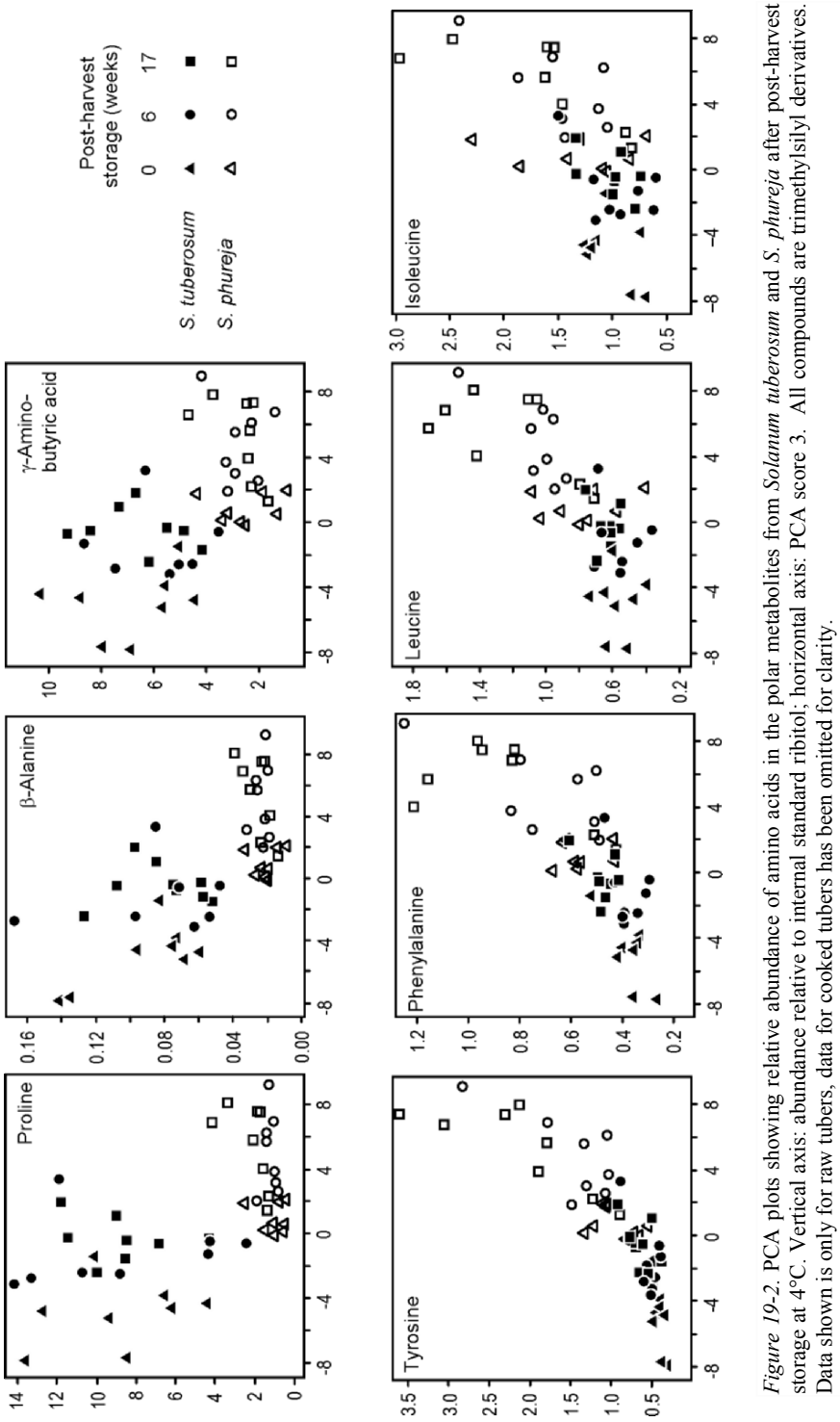

Figure 19-2. PCA plots showing relative abundance of amino acids in the polar metabolites from *Solanum tuberosum* and *S. phureja* after post-harvest storage at 4°C. Vertical axis: abundance relative to internal standard ribitol; horizontal axis: PCA score 3. All compounds are trimethylsilyl derivatives. Data shown is only for raw tubers, data for cooked tubers has been omitted for clarity.

lysine were higher in *S. phureja* line 333/16 only. With the exception of piperidine carboxylic acid (pipecolic acid) and 2,3,4-trihydroxybutyric acid (threonic acid), which were higher in *S. phureja* and *S. tuberosum* respectively, the levels of the other metabolites were similar in both species.

When data for raw tubers alone was considered over all storage periods, all four cultivars of *S. tuberosum* could be separated by PCA. The levels of some amino acids were different between the cultivars; M. Piper was high in γ-amino butyric acid and low in proline, P. Dell was high in β-alanine and Cara was low in lysine. Within *S. phureja*, over all storage periods, all genotypes except 257/28 could be separated from the others. 333/16 was higher in some amino acids (alanine and proline in addition to methionine, valine, and lysine), and citric acid was elevated in 333/16 and 337/37, whereas piperidine carboxylic acid was high in 358/23.

The most striking change with storage was an increase in fructose, glucose, and sucrose. This trend was evident for both species, and PCA of the data for raw tubers clearly separated those samples that were not stored at 4°C from those stored for 6 and 17 weeks, which in turn were not clearly separated. This is not surprising as low temperature sweetening is a well documented phenomenon in potato tubers (Brown et al., 1990). Other changes included increases in the levels of aspartic acid and serine, and a decrease in the level of fumaric acid, after storage.

3.2 Non-polar metabolites

Separation of *S. phureja* and *S. tuberosum* was achieved by PCA of data for the relative concentrations of 35 non-polar metabolites in all samples, both raw and cooked and at all 3 storage dates (Figure 19-1B). Levels of the fatty acids *n*-hexadecanoic acid, 15-methylhexadecanoic acid and *n*-heneicosanoic acid were all elevated in *S. phureja* relative to *S. tuberosum* (Figure 19-3).

On analysis of data for raw tubers, all four cultivars of *S. tuberosum* could be clearly separated by PCA over all storage periods. Among the fatty acids, 15-methylhexadecanoate was low in Cara, *n*-octadecanoate was high in Cara and low in Ailsa, and *n*-hexacosanaote was high in M. Piper. Among the straight chain alcohols, *n*-heptacosanol and *n*-nonacosanol were elevated in M. Piper whereas *n*-heneicosanol was high in Cara and low in Ailsa and M. Piper respectively. The separation between *S. phureja* genotypes was less distinct than with *S. tuberosum*. Over all dates, 337/37 and 358/23, but not 257/28 and 333/16, occurred as discrete groups. *n*-Hexadecanoic acid was particularly high in 358/23, and the order of abundance of 15-methyl hexadecanoic acid was 358/23>337/37>257/28>333/16.

For both *S. phureja* and *S. tuberosum,* separation according to storage date was evident at least for 0 and 17 weeks storage at 4°C. None of the

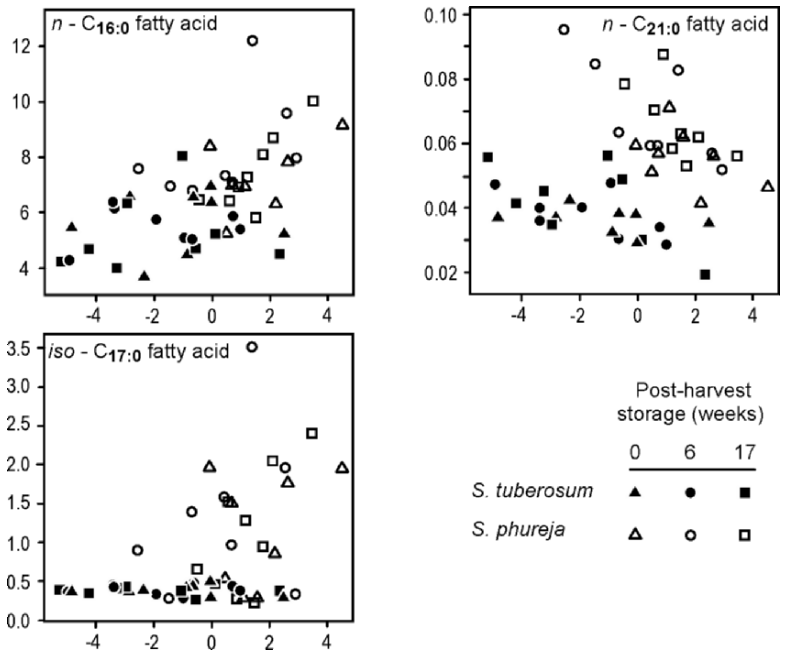

Figure 19-3. PCA plots showing fatty acid levels (as methyl esters) in the non-polar metabolites from *Solanum tuberosum* and *S. phureja* after post-harvest storage at 4°C. Vertical axis: abundance relative to internal standard methyl nonadecanoate; horizontal axis: PCA score 2.

metabolites showed any striking difference in relative abundance between the two storage dates. The levels of *n*-octacosanoic acid tended to decrease with storage in *S. tuberosum*, and in *S. phureja*, *n*-tricosanoic acid tended to increase. There was no evidence for a decrease in linoleic acid and an increase in α–linolenic acid with storage as observed in a previous targeted study on the fatty acids of the same materials (Dobson et al., 2004).

3.3 Volatiles

S. phureja and *S. tuberosum* were separated by PCA of the total ion count area percent compositions of 83 volatiles from cooked tubers (Figure 19-1C). The levels of some *br*-aldehydes (2-methylpropanal, 2-methyl-butanal, 3-methylbutanal), 3-methylthiopropanal, methyl esters of short-chain *br*-acids (2-methylpropanoic acid methyl ester and 2-methylbutanoic acid methyl ester), four sesquiterpenes and several other volatiles (2-methyl-furan, methanethiol, 2-butanone and 2-hydroxybenzoic acid) were higher in *S. phureja*. Hexanal and 2,3-pentadione were elevated in *S. tuberosum*. PCA plots for some of the compounds elevated in *S. phureja* are shown in Figure 19-4.

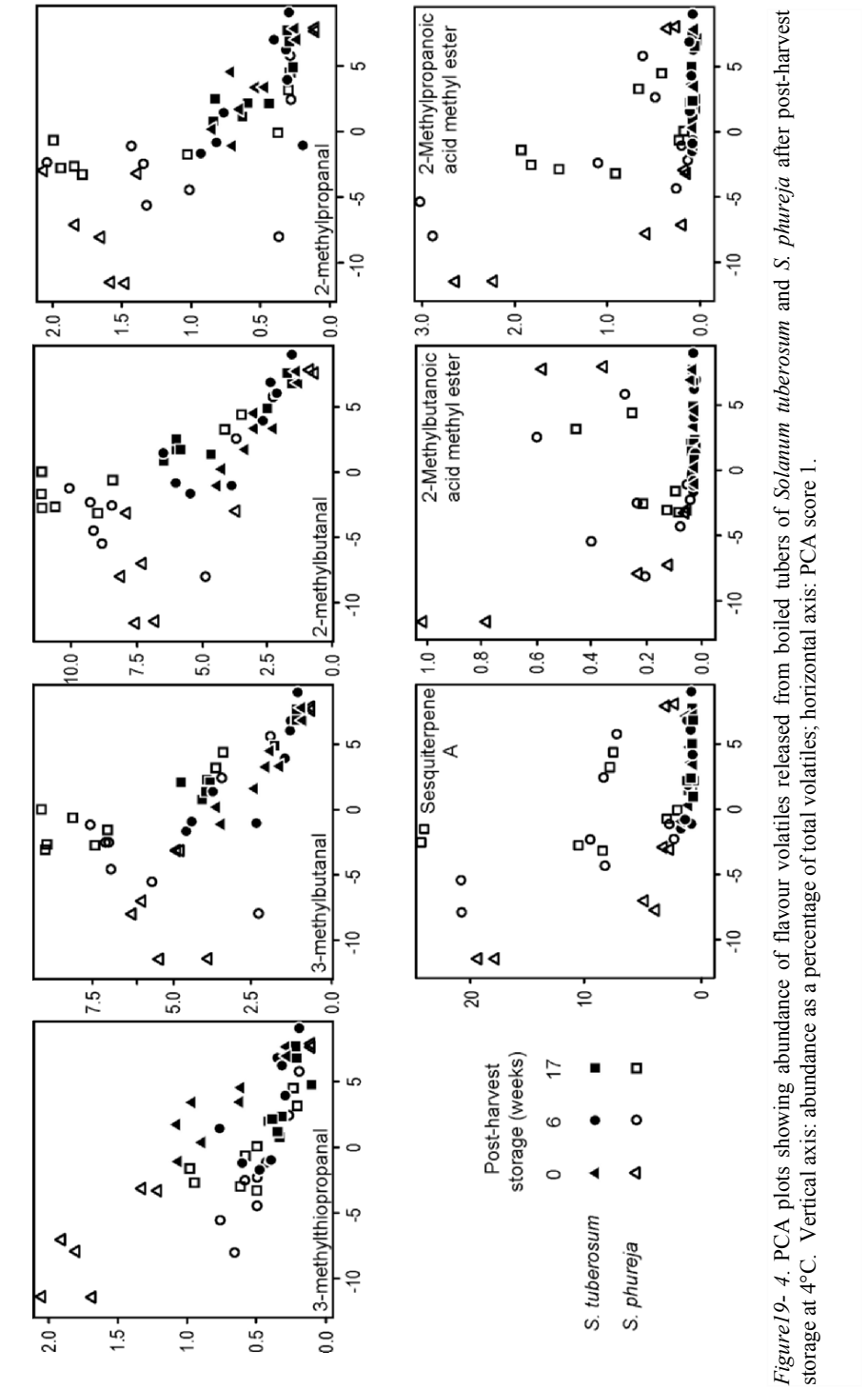

Figure19- 4. PCA plots showing abundance of flavour volatiles released from boiled tubers of *Solanum tuberosum* and *S. phureja* after post-harvest storage at 4°C. Vertical axis: abundance as a percentage of total volatiles; horizontal axis: PCA score 1.

All genotypes of *S. phureja* could be separated by PCA, even when all storage times were considered together. Levels of pentanal, 2-methyl-propanoic acid methyl ester and two of the sesquiterpenes were highest from 333/16. Several aldehydes from 337/37 (hexanal, 5-methylhexanal, and 2,4-heptadienal) were elevated relative to the other genotypes, whereas other aldehydes (2-methylpropanal, 2-methylbutanal, 3-methylbutanal, 2-propenal, 2-methyl-2-butenal, 3-methylthiopropanal, and benzaldehyde) and some other volatiles (2-methylfuran, methanethiol, carbon disulfide, and 2-butanone) were low. 2-Pentylfuran was high from 358/23 and 257/28, whereas two aldehydes (nonanal and decanal) and two alcohols (3-methyl-1-butanol and 2-methyl-1-butanol) were higher in 257/28 and 358/23, respectively.

The unstored cultivars of *S. tuberosum* were all separated by PCA and when all storage periods were considered together, Ailsa and Cara, but not P. Dell and M. Piper, were readily distinguished. The proportions of hexanal, 2-pentenal, and 2,3-pentadione decreased in the order Ailsa > Cara > M. Piper and P Dell, whereas the reverse order was evident for 3-methylbutanal, 2-methylbutanal, 2-pentylfuran, and methanethiol. 3-Methyl-1-butanol and 2-methyl-1-butanol were higher in P. Dell and M. Piper and lower in Ailsa and Cara. Decanal was particularly low in Ailsa whereas a sesqiterpenoid was high in P. Dell.

Considering both species together there was clear separation by PCA according to storage date. Samples stored for 0 and 17 weeks were well separated and those stored for 6 weeks were intermediate in position. The proportions of some alkanes (nonane, decane, and undecane) were higher at 17 weeks, whereas others, of longer chain length (tetradecane, pentadecane, hexadecane, heptadecane, and octadecane), together with some aldehydes (heptanal, undecanal, 2-heptenal, 2-octenal, 2,4-nonadienal, and 2,4-decadienal) were higher at 0-week storage.

4 CONCLUSIONS

It is of interest to catalogue the differences in relative abundances of metabolites between species and among cultivars or genotypes, and changes with duration of storage. Some of these differences, notably the increase in sugars with storage, are well documented, but the majority are not. The real challenge is to identify relationships between different metabolites in terms of changes in metabolic pathways (Figure 19-5). There is a relationship between the elevated abundance of certain *br*- amino acids in tuber of *S. phureja* relative to *S. tuberosum* and similarly elevated levels of short-chain *br*-aldehydes and methyl esters of short chain *br*- acids in the volatile profiles from *S. phureja*. The aldehydes are generated from the amino acids *via* the

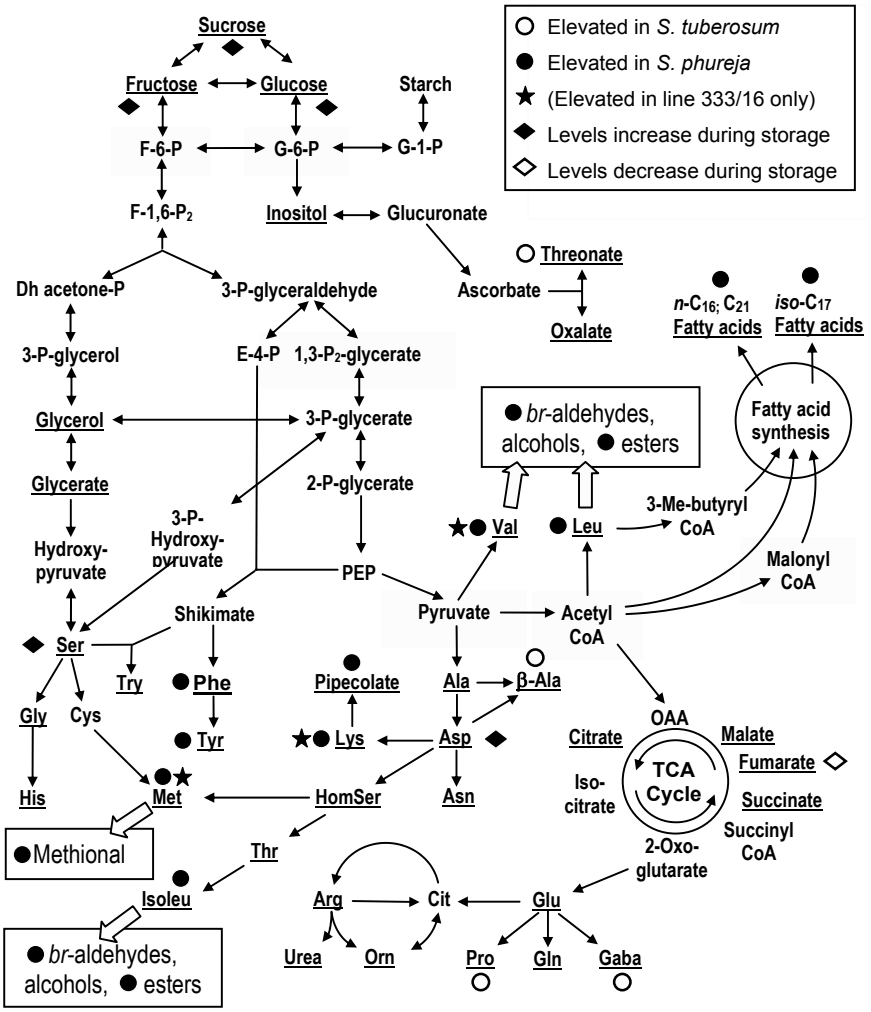

Figure 19-5. Simplified schematic representation of interrelationship between polar and non-polar metabolites isolated from tubers of *Solanum tuberosum* and *S. phureja*. Individual metabolites showing differences in abundance between *S. tuberosum* and *S. phureja* are indicated by open and closed circles. Metabolites which show changes in abundance during storage at low temperature are indicated by closed and open diamonds. Several of the metabolites shown are the source of a number of volatile flavour-related compounds generated when tubers are boiled. These volatiles, shown in the outlined boxes, and their precursor metabolites were relatively more abundant when sampled from tubers of *S. phureja.*

Strecker reaction (Shepherd et al., 2007) and the methyl esters are derived from the equivalent branched acylCoA thioester derivative which in turn is derived from an amino acid. 2-Methylbutanal and 2-methylbutanoic acid

methyl ester, and 2-methylpropanal and 2-methylpropanoic acid methyl ester are derived from isoleucine and valine, respectively, and all these compounds tend to be more abundant in samples from *S. phureja* (valine is elevated in genotype 333/16 only). 3-Methylbutanal and 3-methylbutanoic acid methyl ester are derived from leucine but although the amino acid and aldehyde were elevated from *S. phureja*, the methyl ester was not detected in either species. However, 15-methylhexadecanoic acid, which is derived from 3-methylbutyryl-CoA, the acyl starter unit used during formation of *iso*-branched odd chain fatty acids by the synthesis *de novo*, was more abundant in tubers from *S. phureja*. There were no differences between the species in the abundance of the *br*-alcohols 2-methyl- and 3-methylbutanol which also originate from isoleucine and leucine respectively. For individual genotypes different patterns are evident. For example, the levels of aldehydes in *S. phureja* 337/37 were lower than those of the other *S. phureja* genotypes and were comparable with the levels in *S. tuberosum*.

The increased abundance of *br*-aldehydes and *br*-short-chain esters in the volatile profiles from the four genotypes of *S. phureja* may be significant factors in their favourable flavour assessment by specialist taste panel.

ACKNOWLEDGEMENTS

The authors acknowledge the support of the Scottish Executive Environment and Rural Affairs Department.

REFERENCES

Brown, J., Mackay, G. R., Bain, H., Griffiths, D. W., and Allison, M. J., 1990, The processing potential of tubers of the cultivated potato, *Solanum tuberosum* L., after storage at low temperatures. 2. Sugar concentration, *Potato Res*. **33**:219-227.

De, Maine, M. J., Lees, A. K., Muir, D. D., Bradshaw J. E., and Mackay, G. R., 2000, Long-day adapted phureja as a resource for potato breeding and genetic research, In: *Potato, Global Research and Development-Volume1*, S.M.P. Khurana, G.S. Shekhawat, B.P. Singh, and S.K. Pandey, eds., Indian Potato Association, Shimla, India, pp. 134-137.

Dobson, G., Griffiths, D. W., Davies, H. V., and McNicol, J. W., 2004, Comparison of fatty acid and polar lipid contents of tubers from two potato species, *Solanum tuberosum* and *Solanum phureja*, *J. Agric. Food Chem.* **52**:6306-6314.

Roessner, U., Wagner, C., Kopka, J., Trethewey, R. N., and Willmitzer, L., 2000, Simultaneous analysis of metabolites in potato tuber by gas chromatography-mass spectrometry, *Plant J.* **23**: 131-142.

Shepherd, T., Dobson, G., Marshall, R., Verrall, S., Conner, S., Griffiths, D. W., Stewart, D., and Davies, H. V., 2007, Profiling of metabolites and volatile flavour compounds from *Solanum* species using gas chromatography-mass spectrometry, *Proceedings of the Third International Congress on Plant Metabolomics*, Ames, Iowa, June 2004.