Grower Summary

PE 009

TOMato COMpositional studies to identify and quantify bioactive nutrient associated with tomato fruit quality.

Final 2013

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Industry Representative:	Bernard Sparkes, Melrow Salads Ltd
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Headline

- Concentrations of bioactive compounds reported in this study are in line with those quoted in the scientific literature, if not higher. The nutritional value of UK produced tomatoes has not therefore been compromised by modern production practices, contrary to some claims.

- Similar levels of bioactive nutrients were found in UK grown fruit produced either conventionally or organically.

- Speciality varieties are of distinctive flavour and appearance and may also have distinctive nutrient profiles. This provides opportunities for new product development.

Background

Tomato fruit deliver essential nutrients for the human diet. They provide ready sources of vitamins A, C, E and K, minerals including potassium (K) and iron (Fe) and the lipophilic antioxidant lycopene. Complementary hydrophilic antioxidants such as phenylpropanoids and flavonoids are also present. There is a wealth of scientific evidence that now exists to corroborate that the consumption of fruits and vegetables is beneficial to human health. Previous HDC research (PC 167) showed British grown tomatoes contained significantly higher concentrations of lycopene than those anticipated for fresh tomatoes generally, and imported long shelf-life tomatoes in particular. The present study analyses the bioactive content of tomato fruit and project aims were:

- To update information on the range of nutrients found in UK marketed fresh tomato fruit and imported long shelf-life fruit of known provenance

- To report on the quality of British grown fruit with respect to nutritionally related phytochemicals

The specific objectives were:

1. To compare bioactives present in UK tomato fruit cultivated in different locations and at different times in the season.
2. To compare bioactives present in fruit following conventional and organic cultivation methods.
3. To compare UK and non UK (Spanish and Dutch) cultivated fruit from the same varieties.

4. To perform analyses of bioactives present in novelty varieties compared to classic varieties marketed in the UK.
5. To investigate the effect of the ripening inhibitor gene (*RIN*) in the heterozygous state on bioactive content. A variant of this gene is frequently used in commercial lines because it can confer improved shelf properties.

An audit trial of fruit supply and workflow through the sample preparation and analysis has been provided in the methods of the Science Section of this report. The detailed sampling performed indicated that there was a very high level of consistency and reproducibility among the metabolites analysed in the fruit sampled. These data suggested that the growth and production regimes used, deliver fruit products with consistent metabolite levels between batches/environmental conditions. On average only a 10% coefficient of variation in metabolite content was observed. The sampling of the UK crops was robust and controlled at the point of harvest. Although the Spanish and Dutch sourced varieties were not collected at the point of harvest, the reproducibility of the data indicates that sampling from the trays containing many fruit provides an effective and comparable approach from which valuable data can be extrapolated. This suggests that simpler sampling regimes may be suitable for future research projects on tomato metabolites.

The bioactive compounds compared were:

- Vitamin C
- Total antioxidants
- Phenolics; flavonoids (naringenin and chalcone-naringenin) and phenylpropanoids (chlorogenic acid and rutin)
- Carotenoids (phytoene, phytofluene, lycopene, ß-carotene, lutein) and Vitamin E, (α-tocopherol)

Summary

Objective (1): The effect of (i) sampling early and late season and (ii) location on the content of bioactives in tomato fruit

Three common commercial cultivars were chosen to assess compositional differences between varieties, a classic round (cv. Elegance), a baby plum (cv. Angelle) and a cherry on the vine (cv. Piccolo). To ensure statistical robustness a minimum of six biological replicates (36 fruit sampled from different trusses) were taken per cultivar/treatment. Representative

samples were taken in May 2012 (early) and September 2012 (late). Samples were taken from two production sites located in the UK, designated site A and B.

The data generated suggest that there is a difference between the content of bioactives found in the fruit at different times in the season (Table 1).

Table 1. Summary of increases in bioactives from fruit sampled early or late in the season.

Time of sampling	
Early	Late
-	Lycopene
-	Tocopherol
-	Rutin
-	TEAC

The table represents general trends across the eleven varieties sampled. The quantitative changes found in individual varieties are detailed under the Science Section of this report.

In the case of carotenoid pigments, the content of lycopene present in the ripe fruit was generally higher in the later harvest in the season. For example the range of lycopene contents in the early season samples was determined as 187μg/gDW (Piccolo) to 442μg/gDW (Island Beauty) compared to the later season which ranged from 354μg/gDW (Piccolo) to 573μg/gDW (Island Beauty). In general the varieties showed a consistent 1.3 to 2.0 fold increase from the early to late season lycopene content. Likewise vitamin E content (tocopherols) also increased in the fruit derived from the harvest later in the season. Again typically the increases ranged from 1.3 to 2.0 fold.

One explanation for this occurrence could be that during the start of the production season the plant is channelling a greater proportion of energy into vegetative growth compared with fruit production. The increased light incidence over the season could also be a contributing factor to these findings. The differences associated with phenolic contents did not appear to be linked directly to the early and late timings of harvest but more to varietal differences. However the content of rutin in the fruit did appear to be influenced by the environment. Rutin is found in the peel of tomato fruit and acts as a protectant from light incidence and could explain why its levels are influenced by the timing of harvests within the season.

No strong trends existed between vitamin C and the timing of harvest or the variety. This suggests that in the case of vitamin C the genetic effects are greater than the environment. The total antioxidant capacity of the fruit, as measured by the TEAC assays was greater in

the later season fruit and this finding correlates with the increased lycopene and tocopherol fruit content later in the season.

In general the bioactive content of the tomato fruit was not influenced by production site and a robust reproducibility was achieved between the two sites. However, some differences were found between specific individual components of the different bioactive classes determined. Notably an increase in the lycopene content of Piccolo grown at site B occurred (Table 2).

Table 2. Increases in bioactives at Site B compared with Site A at early and late sampling.

Time of sampling	Piccolo	Angelle
Early	Chalcone-naringenin Lycopene	Chalcone-naringenin
Late	Lycopene	Tocopherol

Tocopherol levels were higher in the Angelle variety grown at site B compared to that from site A. The presence of the flavonoid chalone-naringenin was found to be unique to fruit generated at site B. This compound functions as a protectant against light incidence. Overall the production location did not affect the TEAC levels or vitamin C content. It is conceivable that an increased light incidence could have been associated with site B compared to site A, and may have some bearing on these findings. Further acquisition of climatic parameters over the season could clarify this hypothesis.

The use of different genotypes, good biological replication and analysis performed concurrently by one analyst adds robustness and accuracy to the dataset. However, the sampling of more stages across the season would help define the precise timing of these seasonal trends and differences between production sites.

Objective 2: The effect of conventional and organic cultivation on the bioactive content found in ripe tomato fruit

The varieties Piccolo, Angelle and Green Tiger, were sampled from two production systems, conventional hydroponic (stonewool and coir) and organic soil-grown. The analysis of multiple varieties harvested early and late in the season, across two production sites indicated that conventional and organic cultivation practices had no bearing on the bioactive content of the fruit, an anomaly being the Green Tiger variety which contained a high lycopene content when cultivated organically. However, unambiguous conclusions could not be made in this case because the production sites were different for the conventional and organic samples for this variety and an assessment of stage of ripeness was not

straightforward with red ripe being impossible to define in this 'green' variety. Previous research (HDC PC 167) showed the stage of fruit ripeness to be critical in resultant lycopene concentration.

Objective 3: A comparison of bioactive content found in UK, Spanish and Dutch source tomato fruit

Due to availability, tomato varieties from three countries were sampled on different occasions, Piccolo, Angelle and Elegance sourced from Spain were compared to their UK counterparts cultivated at site A for the early season date only. Comparisons with Dutch fruit were made with the Elegance variety, but only for the late season date. Any comparisons should therefore be made with caution.

Within the sample set studied (Piccolo, Angelle and Elegance varieties) there is a trend towards an increased (up to 2-fold) content of lycopene, associated with ripe fruit from UK source tomato varieties (Piccolo and Angelle) compared with their Spanish comparators (Piccolo and Angelle). Dutch sourced variety Elegance showed little difference in lycopene compared to the UK equivalents for the same sampling date. In the case of tocopherol content the trend was clear and suggested that the Spanish derived fruit contained more tocopherol (up to 2-fold more), though there were differences between varieties. Angelle and Elegance sourced from Spain contained more phenolic compounds (up to 2-fold). However, despite these changes in carotenoid and phenolic contents the total antioxidant content did not change. No variation in Vitamin C content relating to source of fruit was found. The use of identical varieties suggests that the changes arising are due to a combination of effects such as sampling, stage of ripeness, growth conditions in Spain and Holland and transportation through the supply chain, not the genetic or biochemical diversity, although the varieties where not genotyped at the molecular level in this study.

Objective 4: Comparison of bioactive content found in novelty varieties with traditional commercial varieties

Dometica was used as a classic reference variety. This variety was compared to Pink Beef, Jack Hawkins, and Island Beauty which have been documented as high lycopene varieties previously, Green Tiger which has a green fruited phenotype with purple stripes, Orange Baby Plum which has an orange fruit colour and Super Sweetini, which is reported to have high umami flavour characteristics, were also included in this experiment. The umami flavour profile may be associated with glutamate content, though analysis of amino acids and their salts was not included in this study.

In this study Island Beauty, Pink Beef, Green Tiger and Jack Hawkins contained more lycopene compared to Dometica. With Jack Hawkins the late season figure was higher, in line with this variety having the highest lycopene content of those included in previous HDC Project PC 167. Compositionally all varieties were similar with the exception of Orange Baby Plum. Here the high phytoene and accumulation of pro-lycopene is a classic profile of the carotene isomerase (*CRTISO*) tomato allele. This means that when a tomato variety contains this variant (mutated gene, typically termed an allele) in the homozygous (or dominant) state then the phenotype (orange fruit) or even chemotype (pigment content in this case) is typically high phytoene and pro-lycopene accumulation. Interestingly, this form of lycopene is common with that found in the body (plasma) and has previously been reported to be more bioavailable[1]. Green Tiger is another example where a different composition is likely, potentially this variety could contain phytonutrients typical of berries (raspberry and blueberry), such as anthocyanins, although analysis for this was not carried out in this study. There has been extensive work to create tomato varieties with these properties using Genetic Modification (GM) technologies[2].

Across the collection of varieties used, the analyses of phenylpropanoids suggested environmental effects appear to influence the phenylpropanoid/flavonoid content dramatically. However, the presence of two phenotypic backgrounds/phenotypes varieties containing chlorogenic acid as the predominant phenylpropanoid and varieties in which naringenin is the predominant flavonoid can be observed within the data especially with large datasets like that used in objective 1. This emphasises why it is good to include several representative varieties in experiments. Higher rutin content was recorded for Super Sweetini than for other varieties but figures in no way approach those reported for plant tissues considered to have high concentrations of this compound, such as buckwheat, green asparagus and apple (peel).

Analysis of the late season harvest solely, suggests that the Pink Beef, Jack Hawkins and Island Beauty varieties are potentially high vitamin C containing tomatoes. The total antioxidant capacity of the novelty varieties was similar to that found in Dometica, although, the novelty varieties were more consistent in their amounts between the early and late season fruit.

Objective 5: The content of bioactives found in "RIN" varieties sourced from Spain

[1] Moxley *et al.* (1999). *FASEB J.* 13:A211
[2] Butelli *et al*. (2008). Nat. Biotechnol. 26, 1301-1308

The varieties bred using the ripening inhibitor gene for longer shelf-life (*RIN*), Justyna and Ninette, used in this study are both from Hazera Genetics. Justyna is a cherry type of 25-35 mm fruit diameter normally sold on the vine and Ninette a red, round variety with a typical fruit weight of 150-220 g and sold loose. They were sourced in December 2012 and analysed separately to the samples assessed under the first four objectives above. This is not ideal as it can increase variability through machine drift or batch variation. In future studies it may be worth considering including a standardized variety with all batches to act as a reference sample so that quantitative levels and amounts relative to a common variety can be made. In comparison to the levels determined in varieties constituting objective 1 lycopene and tocopherol content in the fruit was higher. These findings were particularly impressive in the Ninette variety, which at 873μg/gDW lycopene was approximately 300μg/gDW higher than the Island Beauty variety which contained the highest content in the previous analyses. The tocopherol level also represented a 2-fold increase in this variety.

In contrast phenolic contents (phenypropanoids and flavonoids) were lower in these varieties, in some cases an order of magnitude less compared to contents ascertained with varieties analysed in objective 1 and 4 harvested later in the season. The vitamin C and total antioxidant activities were similar between the two varieties analysed and within the range of contents/activity determined.

A comparison with published reference levels

In the present study we have prepared the tissue by freeze-drying as this mode of preparation eliminates variation from water content. It is also beneficial for the extraction of hydrophobic molecules, improves handling properties and stability over time. This approach follows the recommendation for reporting metabolite data reported within the scientific community (Fernie et al., 2011, Recommendations for reporting metabolite data, Plant Cell, 23, 2477-2482). These methods are not directly comparable to those previously used in MacCance and Widdowson's, 2004, "The composition of Foods": Summary Ed 6th, Cambridge: Royal Society of Chemistry or USDA National Nutrient Database for Standard Reference, Release 20. In addition we have only performed two sampling regimes each with analysis; no standardised varieties were used between those published and the present set. However, comparisons have been made by converting the datasets to amount (mg) per gram Fresh Weight (see Table 21). These values indicate very strong agreement with those quoted by the USDA and MacCance and Widdowson for lycopene. β-carotene content was greater in this present study delivering twice the proportion of provitamin A; lutein was also greater in the present study. The levels obtained in this study are also in line with the

Moneymaker, Ailsa Craig varieties and distribution across introgression and recombinant inbred collections analysed over the last decade by the analysts. Vitamin E displayed clear agreement with that quoted in the databases and the present study. In the case of vitamin C there was a wider range of levels in the UK crops with the upper level being comparable to that found in the databases.

Annex 1 of the Science Report contains the supplementary information to facilitate the calculation of bioactive content on a FW basis. However, it must be emphasized that these are retrospective calculations, as extractions were performed on freeze-dried material. This approach was used as it eliminates error, cost and environmental impact due to increased volumes, matrix effects, non-optimal solvents, differential and inefficient metabolite extraction and incomplete and irregular homogenization. The use of freeze-dried material has limited analytical error occurring collectively this allows greater confidence in the data and conclusions drawn from the data. Extractions performed on fresh material are affected by fruit texture which in turn has a bearing on bioaccessibility. Any conversion of the data back to the original fresh weight must therefore bear these limitations in mind and be treated with caution.

Conclusions

- Concentrations of bioactive compounds reported in this study are in line with those quoted as standard data in the scientific literature, if not higher. This suggests that the nutritional value of UK produced tomatoes has not been compromised by modern production practices, and may actually be enhanced in respect of some bioactives.

- Similar levels of bioactive nutrients were found in the three varieties of UK grown fruit produced either conventionally or organically. An apparent increase in lycopene in one variety (Green Tiger) grown organically *may* be explained by other factors.

- Increased bioactives content was found in ripe fruit sampled later in the season (September) compared with the early sample (May).

- Significantly higher lycopene content was found in UK cultivated tomatoes compared to the same varieties grown in Spain.

- Phenylpropanoid (flavonoids) and tocopherol content of some varieties was higher in Spanish cultivated tomatoes compared with the same varieties grown in the UK.

- Analytical results in this study are expressed as a proportion of the tissue dry weight, after removal of water by freeze drying of the samples. This needs careful interpretation in relation to expressing results in the fresh product.

- It is logical to assume that varieties with a high dry matter content, which are generally smaller fruited types such as cherry and baby plum varieties Piccolo and Angelle, will contain relatively higher nutrient concentrations in the fresh product. Since these varieties are generally regarded as having an enhanced flavour and are more attractive to younger consumers, this presents obvious marketing and promotional opportunities.

- Some varieties exhibit distinctive compositional profiles, such as Green Tiger, which could contain phytonutrients typical of berries (raspberry and blueberry), such as anthocyanins, although analysis for this was not carried out in this study. Orange Baby Plum contains high phytoene and accumulation of pro-lycopene, a form which is potentially more bioavailable. These varieties are both distinctive in appearance and represent opportunities to develop niche products.

- The high level of consistency and reproducibility among the metabolites analysed in the fruit sampled suggests that cost savings may be achieved in future research projects of this type by reducing the number of sample replicates needing to be analysed for each treatment, at least with fruit of known provenance which is selected and harvested by the researchers themselves and stored under identical conditions.

- The critical relationship between stage of fruit ripeness and levels of metabolites such as lycopene which has been established in earlier research, such as HDC Project PC 167 *Tomatoes: preliminary investigation of the effects of cultivar, stage of harvesting and post-harvest storage on fruit lycopene content.* (1999), which suggests that a more objective assessment of stage of fruit ripeness at analysis is required to ensure meaningful and reliable comparisons. PC 167 found a significant increase in lycopene concentration of fruit retained for a further 7 days at room temperature beyond colour stage 9, the normal commercial colour chart assessment of full ripeness. A 69% increase in lycopene concentration on a fresh weight basis for cherry tomato cv Favorita was recorded for instance. It is proposed that a standardised protocol should be considered for fruit maturity at analysis. Assessment of this for non-red varieties or those with a patterned appearance, such as Green Tiger, present particular

difficulties in this respect. It is possible that concentrations of other metabolites may be declining by Colour Stage 9 + 7 days and this would need to be taken into account in agreeing any protocol.

- Analyses of the mineral element and amino acid content of samples should be considered for future research relating to nutritional status of tomato fruit.

Financial Benefits

The bioactive compositional information provided will help the UK tomato industry promote its products and develop new and distinctive ones with a range of enhanced nutritional composition.

Action Points

- If growers want to focus on nutrient dense tomato fruit then specific varieties can be bred, all the tools are available to achieve this goal.

- Orange Baby Plum and Green Tiger varieties are worthy of further investigation into potential health benefits.