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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headlines

- 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.

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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

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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

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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

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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.

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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

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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

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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.

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- 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

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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.

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2. SCIENCE SECTION

2.1 Introduction

Tomato fruit deliver essential nutrients for the human diet and form the basis of a multi-billion $ global industry. Tomato (*Solanum lycopersicum*) is the most important fruit crop in the world by volume consumed, with annual production of 150 million metric tons. Tomatoes are high value products with an annual value in 2009 of around $32B (faostat.fao.org) covering both processed and fresh products. They are a major component of healthy diets and provide ready sources of vitamins A, C, E and K, minerals including K and 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 (www.ifava.org/science). These benefits have been attributed to the presence of health promoting phytochemicals or "bioactives" in the food matrix. Healthy eating impacts on healthy ageing and therefore this will be critical especially in developed countries where, in the future, health services could be overwhelmed by increasing costs of care for the elderly. This has led to the UK and other governments recommending five portions of fruits and vegetables per day (www.doh.gov.uk/fiveaday). In the majority of cases, these recommendations are not achieved. The challenge, particularly, in Western societies, is to deliver to the consumer better tasting, more nutritious tomatoes and other fruit which have a prolonged shelf-life at a cost easily afforded by the majority of consumers. The most important quality traits in tomato are colour, texture, flavour and nutritional content. The compounds responsible for the red and orange colours are carotenoid and flavonoid pigments both of which also contribute to the health promoting properties of the fruit and can provide precursors of flavour volatile compounds. Texture can also impact on taste, the release of nutrients and perhaps most importantly shelf-life. Fresh tomato consumption in the UK is relatively low compared with other countries however, such as those in southern Europe, which indicates the potential for increased consumption of fresh, flavoursome, nutritious products in this country. It is feasible that highly flavoured products and those with a distinct aroma profile, such as the speciality tomato types which the British industry has increasingly adopted over the past 10 years, may also have high nutrient levels, for reasons such as their high dry matter content and deep colour. Many are also smaller fruited types and the combination of these factors makes them relatively more attractive to children and therefore of particular interest and relevance in children's diets.

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In addition to the wide range of nutrients in tomatoes, they represent the primary source of certain individual nutrients in the diet, the carotenoid lycopene being the prime example. In a typical western diet, 90% of lycopene ingestion derives from tomatoes. One research project (Hayman. HDC PC 167, 1999) showed British grown tomatoes to contain significantly higher concentrations of lycopene than those anticipated for fresh tomatoes generally, and of samples of imported long shelf-life tomatoes in particular.

The present study analysed 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 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. In the heterozygous (*RIN rin*) state two variants of the gene product will exist, the wild type and a variant (termed allele), the latter will confer the desired properties. In the homozygous (*RIN RIN*) state the effects on the phenotype are stronger.

2.2 Materials and methods

(i) ***Cultivation and experimental design.***

Three common commercial cultivars were chosen to assess compositional differences between varieties. These included the classic round (cv. Elegance), a baby plum (cv.

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Angelle) and a cherry on the vine (cv. Piccolo). These varieties were common to both production site A and B, hence providing two locations with the same varieties. To ensure statistical robustness a minimum of six biological replicates were performed per cultivar/treatment.

The sampling procedure was devised to take into account the different shape and size of the glasshouse compartments. The scale of the trial plan was varied depending on these variables and for example, for one cultivar the size of one block was 3 rows of 10m length whilst for another there were 10 rows by 40 m. Within each plot 6 tomatoes were taken from different trusses which would then be combined from the same number plot in the other 5 blocks to make 1 biological sample (6 individual fruit) with 6 replicates per treatment. So in summary one biological sample comprised of six pooled fruit per block generating six 'biological samples'. From each biological sample a minimum of three technical replicates were analysed.

The full detail of the sampling programme is given below. For these samples fruit were picked at the standard colour stage (e.g. stage 5) and then treated as if they progressed through the supply chain to the supermarket shelf with analysis taking place at the point of consumption stage. Fruit from Spain and Holland were sampled from imported product available in the packhouse of site B, aiming for consistency of stage of ripening with the samples collected from UK sites. Handling of this imported fruit was then identical to the UK origin samples but transportation to the point of sampling was a point of difference between these sample sets.

Proposed Expt 1*.*

To evaluate the impact of season by comparing an early and late sample, as follows:

3 varieties x 2 locations x 2 dates x 6 replicates = 72 samples.

Modifications:

Due to availability 13 samples were collected for both the early (May) and late season (September) stages. These comprised of 8 varieties over both early and late season with a further two varieties repeated at each location as follows:

8 varieties x 2 dates x 6 replicates = 96 samples.

2 varieties x 2 locations x 2 dates x 6 replicates = 48 samples.

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This analysis was accompanied by preliminary analysis to establish inter fruit variation 6 fruit x 3 replicates = 18 samples

Proposed Expt 2.

The impact of production systems e.g. conventional vs organic by comparing cv. Piccolo from organic and conventional systems at the same location, as follows:

1 variety x 2 systems x 1 site x 2 dates x 6 replicates = 24 samples.

Modifications:

The varieties, Piccolo, Angelle and Green Tiger, were sampled from the conventional and organic systems at production sites A and B sites as follows:

2 varieties x 2 systems x 1 site x 2 dates x 6 replicates = 48 samples.

1 variety x 2 systems x 1 site x 2 dates x 6 replicates = 24 samples

Proposed Expt 3.

The impact of country of origin via comparison of non-UK and UK fruit taken from the supermarket shelf by selecting a common variety determined by availability with the aim of comparing:

3 countries (UK, Spain, Holland) x 1 supermarket x 2 dates x 6 replicates

= 36 samples.

Modifications:

Due to availability, tomato varieties from three countries were sampled on different occasions taking fruit from a commercial packhouse:

(UK and Spain) 2 varieties x 2 dates x 6 replicates = 24 samples

(UK and Holland) 1 variety x 1 date x 6 replicates = 12 samples

Proposed Expt 4.

The impact of novelty varieties with potentially different compositional spectrum by comparing a yellow cherry (cv Orange Baby Plum), cv. Green Tiger and a fruit bred specifically for a health promotion (not yet named), giving:

3 varieties x 6 replicates = 18 samples.

Modifications:

Extra varieties were included as follows:

7 varieties x 2 dates x 6 replicates = 84 samples

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Additional experiment.

Expt 5. Compare two "*RIN*" long shelf-life varieties Justyna and Ninette sourced from Spain i.e.:

2 varieties x 1 location x 6 replicates = 12 samples

Collectively this adds up to 366 samples for analysis through four analytical platforms with a minimum of three technical replications equals 4,392 determinations.

(ii) Material collection and preparation

An inventory of the samples analysed, their source, harvest/sampling time point, and preparation through the workflow is provided in **Tables 3**, **4** and **5**. Following harvest, tomato fruit were left for three days at 10^{o}C. The fruit were then weighted, cut in half, frozen and placed into the freeze dryer for another three days. After this period the fruit were completely dry and the material then ground in a TissueLyser LT (Qiagen). The resulting powder was used in further analysis.

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Table 3. A summary of the early season samples collected and analysed.
BC = Coir & RW = Rockwool growing media. A = production site A, and B = production site B.
Immediately following harvest, all samples were stored for 3 days at 10°C and were then freeze dried for 3 days with analysis following during the period June to August 2012.

Variety	Mode of cultivation	Source	Site / Collection date	RHUL ID
Piccolo	conventional	Spain	A / May 2012	R1
Piccolo	conventional	UK	B / May 2012	R2
Piccolo	organic	UK	B / May 2012	R3
Piccolo	conventional (BC)	UK	A / May 2012	R4
Piccolo	conventional (RW)	UK	A / May 2012	R5
Angelle	conventional	Spain	A / May 2012	R6
Angelle	conventional	UK	A / May 2012	R7
Angelle	conventional	UK	B / May 2012	R8
Angelle	organic	UK	B / May 2012	R9
Elegance	conventional	Spain	A / May 2012	R10
Elegance	conventional	UK	A / May 2012	R11
Green Tiger	conventional	UK	A / May 2012	R12
Green Tiger	organic	UK	B / May 2012	R13
Pink Beef	conventional	UK	B / May 2012	R14
Jack Hawkins	conventional	UK	B / May 2012	R15
Orange Baby Plum	conventional	UK	B / May 2012	R16
Island Beauty	conventional	UK	B / May 2012	R17
Super Sweetini	conventional	UK	A / May 2012	R18
Amoroso	conventional	UK	A / May 2012	R19
Dometica	conventional	UK	B / May 2012	R20

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Table 4. A summary of the late season samples collected and analysed.
BC = Coir & RW = Rockwool growing media. A = production site A, and B = production site B.
Immediately following harvest, all samples were stored for 3 days at 10°C and were then freeze dried for 3 days with analysis following during the period October to November 2012.

Variety	Mode of cultivation	Source	Site / Collection date	RHUL ID
M82 processing line	conventional	UK	RHUL Sept. 2012	R21
Piccolo	conventional	UK	B / Sept 2012	R22
Piccolo	organic	UK	B / Sept 2012	R23
Piccolo	conventional (BC)	UK	A / Sept 2012	R24
Piccolo	conventional (RW)	UK	A / Sept 2012	R25
Angelle	conventional	UK	A / Sept 2012	R26
Angelle	conventional	UK	B / Sept 2012	R27
Angelle	organic	UK	B / Sept 2012	R28
Elegance	conventional	UK	A / Sept 2012	R29
Elegance	conventional	NL	A / Sept 2012	R30
Green Tiger	conventional	UK	A / Sept 2012	R31
Green Tiger	organic	UK	B / Sept 2012	R32
Pink Beef	conventional	UK	B / Sept 2012	R33
Jack Hawkins	conventional	UK	B / Sept 2012	R34
Orange Baby Plum	conventional	UK	B / Sept 2012	R35
Island Beauty	conventional	UK	B / Sept 2012	R36
Super Sweetini	conventional	UK	A / Sept 2012	R37
Dometica	conventional	UK	B / Sept 2012	R38

Table 5. Additional "RIN" samples supplied.
A = production site A. Immediately following harvest, all samples were stored for 3 days at 10°C and were then freeze dried for 3 days with analysis following during January 2012.

Variety	Mode of cultivation	Source	Site/ Collection date	RHUL ID
Justyna	conventional	Spain	A / Dec 2012	R39
Ninette	conventional	Spain	A / Dec 2012	R40

(iii) Analytical methods: Quantitative analysis of bioactives

Vitamin C determination

The assay method measures both oxidised and reduced forms of ascorbic acid, giving the total vitamin C levels for the sample, as well as the amount of DHA. The use of microplates makes possible the simultaneous analysis of several samples and standards. The microplate has 96 wells, out of which 6 x 3 were for the standard. Each sample took up six wells on the plate. New ascorbate solutions were prepared for each assay.

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Extraction of vitamin C from a dried sample. 50mg of dried tomato fruit powder and ice cold Trichloroacetic acid (600□l) was placed into an micro-centrifuge (2ml). The suspension was mixed by vortexing for 10 seconds, then left on ice for 15 minutes. The sample was centrifuged for 15 minutes at 25,000g and 4^{o}C to clarify. The resulting supernatant was collected for the assay.

Vitamin C concentration measurement using a spectrometric assay. The following solutions were prepared: Sodium ascorbate (1mg/ml) in 6% w/v TCA, dithiothreitol (DTT), 5mM in 0.4M phosphate buffer pH7.4, N-ethyl maleimide (NEM) 0.5% v/v in water, 31% v/v orthophosphoric acid, 0.6% w/v iron chloride, 4% w/v 2,2-dipridyl (in 70% v/v ethanol), 0.4M phosphate buffer pH7.4 and 4.6% w/v thricholoracetic acid (TCA).

From a stock solution of ascorbate standards, the following dilutions were prepared 0.5, 10, 15, 20 and 30 nmol per 20ml. Sample or standard (20 □l) were placed into wells on the microtiterplate. DTT 5mM (in buffer 0.4M phosphate pH7.4) was added to the wells. The plate was covered and incubated for 20 minutes at 37^{o}C. NEM (10□l) was added to each well on the microtiterplate. Samples were mixed and left for 1 minute room temperature. The colour reagent for assays was prepared by mixing 2.75 parts of Solution A to 1 part of solution B. The solutions contained A- Orthophosphoric acid (31% v/v), TCA (4.6 w/v), and iron chloride (0.6 w/v) and solution B- 4% w/v 2, 2-dipyridyl (in 70% ethanol). The colour reagent 80 □l was added to the wells and the standards and samples measured at 550nm using a microtiterplate reader. Values were determined by comparison with dose response curves and the amounts represented as a proportion of the tissue weight analysed.

Carotenoid and tocopherol (vitamin E) determination

Homogenised freeze dried tomato powder (10mg) was weighted into a microcentrifuge tube. Methanol (250μl) was added to the powder, which was then mixed. Chloroform (500μl) (laboratory reagent grade) was added and the suspension vortexed. Samples were then left on ice in darkness for 20 minutes. Tris-HCl buffer (100mM, pH7.5) or water (HPLC grade), 250μl were then added to the suspension and mixed by vortexing before centrifuging the mixture at 12,000 rpm (13,523 rcf) for 5 minutes. The resulting tube contained a phase separation between the non-polar from aqueous solutions. The non-polar chloroform phase containing isoprenoid extracts was on the bottom and was removed and transferred to a new microcentrifuge tube. An additional, 500μl of chloroform was added to the aqueous phase, and a second extraction by vortex and centrifugation was conducted as described above. The pooled chloroform extracts were combined and taken

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to dryness using the EZ-2 plus personal evaporator (GeneVac Ltd) and stored at -20^{o}C until further analysis. HPLC grade ethyl acetate (50μl) was added to the dried isoprenoid extract, to redissolve the material. Injections of 3μl were made onto a C18 BEH column using a UPLC Acquity separation module from Water Ltd. The gradient used to separate the pigments was comprised of A: Methanol/$H_2$0 (50:50) and B: Acetonitrile/Ethyl acetate (75:25), initial conditions were A 30% v/v and B 70% v/v leading to 100% B over 6 min. The flow rate used was 0.6ml/min. The elute was monitored continuously with a Photo Diode Array (PDA) detector from 250 to 600 nm. Identification was carried out by co-chromatography and spectral comparisons between authentic standards, Beta carotene (provitamin A), phytoene, lycopene, lutein and zeaxanthin. Quantification was performed from dose response curves. In cases where greater resolution was required conventional HPLC was used employing a C30 column (YMC, Fischer Scientific, UK) and Alliance separation module. The system is described in Fraser et al. (2000) Plant J., 24, 551-558. Compound confirmation was performed using LC-MS, the chromatographic conditions used were similar to Fraser et al., 2000. Detection was carried out by Atmospheric Chemical Ionisation (APCI) in positive mode with a Bruker MAXIS Q-Tof instrument. Tocopherol determinations were carried using the same separation conditions. Extractions utilised 25mg freeze-dried material and a saponification step performed in 6% KOH at 50^{o}C for 1.5 hours prior to extraction in chloroform. The chromatograms were recorded at 300nm and quantification carried out from dose response curves.

Flavonoids and phenylpropanoids

Freeze-dried material (50mg) was suspended in methanol (1 ml). The suspension was incubated at 90^{o}C for 60 min. After cooling on ice, the suspensions were centrifuged at 3500rpm for 5min. The resulting supernatants were passed through a 0.2 μm filter, taken to dryness. The residue was made up in methanol (50μl). Injections of 3μl were made onto a Van Guard pre-column and BEH C18 resolving column with using a UPLC Acquity separation module. The gradient used to separate the pigments included A: Methanol/$H_2$0 (2%v/v) containing formic acid (0.1% v/v) pH2.5 and B: Acetonitrile, initial conditions where A 90% and B 10% going to 70% A, 30% B over 6 min. The flow rate used was 0.4ml/min. Elution from the column was monitored continuously with a Photo Diode array detector from 250 to 600 nm. Identification was carried out by co-chromatography and spectral comparisons between authentic Chlorogenic acid, chalone-narginenin, narginenin and rutin. Quantification was performed from dose response curves. Compound confirmation was performed using LC-MS; the chromatographic conditions used were similar. The system used comprised of an Electrospray ionisation (ESI) ionisation in negative mode with a

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Bruker MAXIS Q-Tof instrument.

Total antioxidant assays

The following solutions were prepared:

ABTS$^+$ Stock solution: This was prepared by reacting potassium persulphate (2.45 mM, final concentration, 0.662 mg/ml solution (6.62 mg in 10 ml) with the aqueous ABTS diammonium salt [2,29-azinobis-(3-rthylbenzothiazoline-6-sulphuric acid)] $C_{18}H_{16}N_4O_6S_4(NH_4)_2$ 7mM in the dark at room temperature over 12-16 h. The ABTS (7mM) was made fresh by adding 38.4 mg to 10 ml of water. The ABTS$^+$ Stock solution is stable in the dark at room temperature for 2 days.

Working solution: This solution was prepared by diluting the ABTS$^+$ stock solution with ethanol up to an Abs of 0.7 at 734 nm and 30^{o}C.

Trolox: The stock solutions of trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) in ethanol (2.5 mM) were freshly prepared (0.626 mg Trolox/ml solution by adding 15.65 mg of Trolox to ethanol 25 ml).

Working solutions:. Five fresh standard working solutions were prepared daily on dilution with ethanol. The final concentration of the standard Trolox, 0 and 15 μM (on addition of 1ml of ABTS.+ the concentration of the Trolox working solutions decreased 100-fold, since aliquots of 10 μl of the latter were added in the cuvette).

Upon establishing the calibration, samples were analysed with the addition extracts in methanol (10μl) and the reduction in Abs at 734 nm recorded. Quantification was made by comparison to the Trolox dose response curves.

Statistical analysis

Six biological and three technical replications were analysed. Data was represented as an average with the error represented by standard deviation. Typically data was expressed as μg/gDW which is the community standard way of presenting such data for comparisons among varieties. Significance was judged using Student t-test the level of significance was judged by P values in the following range $P<0.05$, $P<0.01$ and $P<0.001$ carried out using EXCEL**.**

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2.3 Results and discussion

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

Experiment 1 was designed to compare the contents of bioactives in ripe tomato fruit harvested early (May) and late (September) in the season. As stated in the methods section this approach comprised of 12 sample sets containing 10 varieties. A subset of this experiment was designed to compare two production sites designated A and B. Prior to undertaking the analysis, inter fruit variation was determined. Bioactive contents between fruit were very similar with a 10% coefficient of variation (CV) determined.

Carotenoid and tocopherol contents: The data generated has been quantified and tabulated (**Table 6)**. The carotenes phytoene, phytofluene and lycopene, the carotenoid β-carotene, the xanthophyll lutein and the vitamin E α –tocopherol were all detected in the ripe fruit from all varieties/treatments. In comparison to fruit harvested early in the season the later harvest generated fruit with significantly ($p < 0.001$ to 0.05) higher lycopene contents between comparative varieties, the only exception being Elegance. The range of lycopene contents in the early season samples was determined as 187μg/gDW (Piccolo, R4) to 442μg/gDW (Island Beauty, R17) compared to the later season which ranged from 354μg/gDW (Piccolo, R24) to 573 μg/gDW (Island Beauty, R36). In general the varieties showed a consistent 1.3 to 2.0 fold increase from the early to late season lycopene content, the exception being Green Tiger (R12 and R31) which showed a 2.6 fold increase in lycopene content between the harvest times. As mentioned elsewhere, the stage of fruit ripeness is critical to lycopene concentration and this factor is difficult to assess in a fruit with a patterned background colour, including green. Jack Hawkins showed a 2.0 fold increase in lycopene between early and late season sampling and this variety showed the highest level of lycopene of the varieties compared in HDC Project 167 (1999). Although there was a trend for increases in phytoene, phytofluene, β-carotene and lutein fruit contents between the early and late harvests none of the increases were statistically significant.

Piccolo and Angelle varieties were used to provide a comparison between production sites. Piccolo harvested in the early season contained very similar levels of phytoene, phytofluene, β-carotene and lutein at both production sites. Lycopene content in the early season in Piccolo was however greater in the batch harvested from site B. The increase being 1.6 fold and was significant with a $p < 0.05$ value. Angelle had comparable lycopene content at both production sites.

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α-Tocopherol (vitamin E) was found in all varieties (samples). As found with the carotenoids, tocopherol content increased in the fruit derived from the harvest later in the season compared to the early harvest. The exceptions to this trend was the Site A Piccolo (R4 and R24) variety where α-Tocopherol levels were the same in both samples and Pink Beef (R14 to R33) which showed a decrease in late season fruit compared to their early counterparts. Typically the increases ranged from 1.3 to 2.0 fold with Angelle (R8 to R24) and Jack Hawkins (R15 to R34) giving 2.5 to 3.0 fold increases respectively.

Angelle fruit contained the same level of tocopherol when harvested early from either sites A or B. In late season an increase of 1.7 fold was found in fruit generated from site B.

Determination of phenolic content: The phenylpropanoid chlorogenic acid, flavonoids rutin, naringenin and chalcone naringenin were determined in ripe fruit from the varieties designated in Expt. 1, over the two sampling stages (early and late season). The data has been summarised in **Table 7**. Early season fruit contained chlorogenic acid ranging from 5μg/gDW (Dometica, R20 & Island Beauty R17)) to 71μg/gDW (Orange Baby Plum, R16,). Determinations from the late season batch ranged from 13μg/gDW (Angelle, R26) to 50μg/gDW (Orange Baby Plum, R35). Overall the range was comparable between the harvests across the season. However, the Piccolo (Site A and B), Angelle (A and B), Orange Baby Plum and Elegance displayed increases in chlorogenic acid present in fruit harvested during the early season, while Green Tiger, Pink Beef, Island Beauty and Dometica had an increase in chlorogenic acid from fruit harvested during the late season. Jack Hawkins and Super Sweetini displayed no changes in chlorogenic levels between sampling regimes. In most cases these trends are significant ($p > 0.01$ to $p < 0.05$), but the overriding observations would link the changes in phenolics content to a biochemical/genetic basis as the profile of phenolics among the population could be grouped into varieties showing high chlorogenic acid or flavonoid.

The levels of rutin ranged from not detectable (Elegance, R11, Pink Beef, R14 and Jack Hawkins, R15) to 11μg/gDW (Super Sweetini, R18) in the early season, those harvested later ranged from 1μg/gDW (Pink Beef, R33) to 13μg/gDW (Super Sweetini, R37). No varieties showed a significant increase in rutin content in the early season fruit compared to the fruit from the later season. However, Angelle (site B, R27), Elegance (site A, R29), Pink Beef (R33) and Island Beauty (R36) contained increased rutin in the late season fruits for example. The significant levels for these increases varied from $P < 0.001$ to $P < 0.05$ but the

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accuracy is misleading as in some varieties the levels of rutin were not detectable. This does not mean that the compounds were not present but beyond the detection limits of the analytical platform (e.g. the system can detect standards in the nmol range). All the rest of the varieties showed an increase or no significant change in their rutin contents between the harvest regimes.

Naringenin contents ranged from 2μg/gDW (Jack Hawkins, R15) to 22μg/gDW (Piccolo site A, R4) in the fruit harvested early in the season and 3 μg/gDW (Angelle site A, R26) to 31μg/gDW (Pink Beef, R33) in fruit harvested later in the season. Green Tiger (R12 and R31), Super Sweetini (R18 and R37) and Dometica (R20 and R38) showed no significant change in levels between sampling periods early and late in the season. Increased naringenin was found in fruit harvested early in the season from the Piccolo (site B, R2 and also site A, R4), Angelle (site A, R7 and also site B, R8), and Orange Baby Plum (R16), the increases were significant as judged by $P < 0.001$ to $P<0.05$. Elegance (site A, R29), Pink Beef (R33), Jack Hawkins (R34) and Island Beauty (R36) contained increased naringenin in fruit harvested later in the season as judged by significance values of $P < 0.001$ to $P<0.05$.

Chalcone-naringenin was not detectable in some varieties both in the early and late harvests. The highest contents in the early season were found in Piccolo (WS, R2), 21μg/gDW and in the late season Orange Baby Plum (R35), 12μg/gDW. The varieties Angelle (site B), and Pink Beef showed no significant change in Chalcone-naringenin contents between harvests. Piccolo site B, (R2) was the only sample with significantly increased ($P<0.05$) Chalcone-naringenin in the early harvest, while Piccolo (A, R24), Angelle (A, R26), Elegance (A, R29), Green Tiger (R31), Orange Baby Plum (R35), Island Beauty (R36), Dometica (R38) and Super Sweetini (R37) all showed increased $P<0.001$ to $P<0.05$, levels in the harvest performed later in the season.

Comparisons between production locations showed some changes particularly in Chalcone-Naringenin levels as this flavonoid was not detected in the Piccolo or Angelle varieties generated early in the season from site A. Later in the season there were no significant differences between phenolic content in the fruit.

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Table 6. Mean Carotenoid and tocopherol contents determined in ripe tomato fruit collected in early or late season (±SD). The representation and statistical treatment of the data is provided in the methods section. All samples were taken from conventional growing systems. A = production site A and B production site B. #[1] = mean of samples grown on two growth media rockwool and coir used at site A. #[2] – the lycopene present was prolycopene not all-trans lycopene.

Variety	UK source	Picking season	RHUL ID	Carotenoids and tocopherols (µg/gDW)					
				Phytoene	Phytofluene	Lycopene	ß-Carotene	Lutein	α-tocopherol
Piccolo	B	Early	R2	199 ± 13	181 ± 6	303 ± 57	188 ± 6	171 ± 1	20 ± 4
		Late	R22	219 ± 4	205 ± 2	460 ± 11	208 ± 2	173 ± 0	31 ± 4
Piccolo	A #[1]	Early	R4	196 ± 7	186 ± 8	187 ± 21	195 ± 6	167 ± 3	26 ± 3
		Late	R24	214 ± 2	200 ± 1	354 ± 18	198 ± 2	170 ± 0	26 ± 3
Angelle	A	Early	R7	191 ± 4	180 ± 2	323 ± 41	182 ± 4	170 ± 1	9 ± 6
		Late	R26	225 ± 3	204 ± 2	430 ± 25	193 ± 4	170 ± 0	12 ± 4
Angelle	B	Early	R8	194 ± 15	180 ± 9	266 ± 84	180 ± 4	170 ± 1	8 ± 4
		Late	R27	207 ± 5	194 ± 3	451 ± 14	199 ± 5	173 ± 0	20 ± 3
Elegance	A	Early	R11	219 ± 12	186 ± 6	408 ± 98	181 ± 4	171 ± 1	13 ± 3
		Late	R29	234 ± 1	205 ± 1	423 ± 12	198 ± 2	174 ± 0	29 ± 4
Green Tiger	A	Early	R12	198 ± 22	186 ± 9	196 ± 18	183 ± 14	185 ± 18	25 ± 9
		Late	R31	229 ± 6	210 ± 3	505 ± 27	212 ± 6	181 ± 0	31 ± 5
Pink Beef	B	Early	R14	230 ± 24	188 ± 10	372 ± 78	188 ± 7	171 ± 1	17 ± 7
		Late	R33	226 ± 4	198 ± 3	417 ± 35	197 ± 4	171 ± 0	14 ± 4
Jack Hawkins	B	Early	R15	193 ± 7	175 ± 3	273 ± 49	175 ± 3	171 ± 1	11 ± 4
		Late	R34	232 ± 3	208 ± 3	548 ± 21	193 ± 2	176 ± 0	34 ± 6
Orange Baby Plum	B	Early	R16	273 ± 28	216 ± 14	250 ± 11 #[2]	172 ± 11	169 ± 1	6 ± 0
		Late	R35	277 ± 7	230 ± 4	390 ± 18 #[2]	187 ± 2	173 ± 0	19 ± 3
Island Beauty	B	Early	R17	208 ± 13	198 ± 11	442 ± 124	196 ± 12	170 ± 1	24 ± 14
		Late	R36	233 ± 4	211 ± 3	573 ± 43	224 ± 5	174 ± 0	37 ± 3
Super Sweetini	A	Early	R18	200 ± 23	175 ± 22	188 ± 16	188 ± 4	174 ± 2	24 ± 5
		Late	R37	229 ± 3	205 ± 2	403 ± 17	221 ± 5	176 ± 0	37 ± 3
Dometica	B	Early	R20	180 ± 7	180 ± 7	232 ± 69	188 ± 13	171 ± 13	15 ± 1
		Late	R38	218 ± 4	202 ± 3	487 ± 27	207 ± 5	174 ± 1	31 ± 7

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Table 7. Phenylpropanoid contents determined in ripe tomato fruit collected in early or late season. The representation and statistical treatment of the data is provided in the methods section. All samples were taken from conventional growing systems. A- production site A and B- production site B. #[1] – mean of samples grown on two growth materials rock wool and coir used at A .

Variety	UK source	Picking season	RHUL ID	Phenylpropanoid (µg/gDW)			
				Chlorogenic acid	Rutin	Naringenin	Chalcone naringenin
Piccolo	B	Early	R2	27 ± 2	6 ± 1	15 ± 3	21 ± 1
		Late	R22	16 ± 0	5 ± 1	8 ± 3	5 ± 0
Piccolo	A #[1]	Early	R4	29 ± 3	6 ± 2	22 ± 4	0
		Late	R24	16 ± 1	4 ± 2	6 ± 0	4 ± 3
Angelle	A	Early	R7	34 ± 7	5 ± 2	13 ± 3	0
		Late	R26	13 ± 3	6 ± 0	3 ± 2	3 ± 2
Angelle	B	Early	R8	49 ± 12	4 ± 1	13 ± 6	6 ± 0
		Late	R27	24 ± 1	8 ± 2	4 ± 2	5 ± 2
Elegance	A	Early	R11	28 ± 1	0	5 ± 0	0
		Late	R29	16 ± 3	2 ± 1	20 ± 4	3 ± 2
Green Tiger	A	Early	R12	10 ± 3	1 ± 0	15 ± 1	0
		Late	R31	12 ± 2	2 ± 0	12 ± 5	1 ± 0
Pink Beef	B	Early	R14	15 ± 0	0	3 ± 0	0
		Late	R33	20 ± 4	1 ± 0	31 ± 11	0
Jack Hawkins	B	Early	R15	15 ± 0	0	2 ± 0	0
		Late	R34	15 ± 4	2 ± 2	18 ± 6	4 ± 3
Orange Baby Plum	B	Early	R16	71 ± 13	7 ± 3	8 ± 4	1 ± 0
		Late	R35	50 ± 2	9 ± 1	0	12 ± 3
Island Beauty	B	Early	R17	5 ± 1	1 ± 0	7 ± 1	0
		Late	R36	18 ± 1	3 ± 1	23 ± 4	6 ± 4
Super Sweetini	A	Early	R18	13 ± 2	11 ± 0	18 ± 3	0
		Late	R37	16 ± 3	13 ± 0	16 ± 1	2 ± 1
Dometica	B	Early	R20	5 ± 1	1 ± 0	8 ± 2	0
		Late	R38	15 ± 1	2 ± 1	12 ± 3	1 ± 1

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Table 8. Vitamin C contents and Total Antioxidant Contents (TEAC) determined in ripe tomato fruit collected in early or late season. The representation and statistical treatment of the data is provided in the methods section. All samples were taken from conventional growing systems. A = production Site A and B = production site B. #[1] = mean of samples grown on two growth media rock wool and coir used at site A.

Variety	UK source	Picking season	RHUL ID		
				Vitamin C mgVitC/100g DW	TEAC μM/mgDW
Piccolo	B	Early	R2	74 ± 23	0.6 ± 0.1
		Late	R22	34 ± 2	1.3 ± 0.1
Piccolo	A #[1]	Early	R4	59 ± 17	1.0 ± 0.1
		Late	R24	39 ± 8	1.4 ± 0.1
Angelle	A	Early	R7	32 ± 13	0.9 ± 0.1
		Late	R26	29 ± 6	1.0 ± 0.2
Angelle	B	Early	R8	38 ± 12	0.7 ± 0.1
		Late	R27	29 ± 4	1.4 ± 0.1
Elegance	A	Early	R11	17 ± 8	0.8 ± 0.1
		Late	R29	39 ± 4	1.4 ± 0.1
Green Tiger	A	Early	R12	41 ± 10	0.7 ± 0.1
		Late	R31	29 ± 4	1.1 ± 0.1
Pink Beef	B	Early	R14	36 ± 10	0.7 ± 0.1
		Late	R33	63 ± 4	1.2 ± 0.2
Jack Hawkins	B	Early	R15	37 ± 18	0.8 ± 0.3
		Late	R34	57 ± 6	1.2 ± 0.1
Orange Baby Plum	B	Early	R16	75 ± 20	0.8 ± 0.1
		Late	R35	31 ± 6	1.6 ± 0.1
Island Beauty	B	Early	R17	25 ± 7	0.5 ± 0.1
		Late	R36	42 ± 8	1.6 ± 0.2
Super Sweetini	A	Early	R18	35 ± 6	0.7 ± 0.2
		Late	R37	31 ± 6	1.2 ± 0.1
Dometica	B	Early	R20	74 ± 29	0.4 ± 0.1
		Late	R38	28 ± 5	1.3 ± 0.1

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Vitamin C and TEAC levels: The vitamin C (VitC) contents and total antioxidant (TEAC) activities found in the ripe fruit generated for Expt. 1 are provided in **Table 8**.

Vitamin C levels in fruit from the early harvest ranged from 17mgVitC/100gDW (Elegance FF, R11) to 74mgVitC/100gDW (Piccolo WS, R2 and Dometica, R20) and fruit harvested later in the season 28mgVitC/100gDW (Dometica, R38) to 63mg VitC/100gDW (Pink Beef, R33). No strong trends existed between vitamin C and the timing of harvest or the varieties. A comparison between locations (site A and B) for the Piccolo and Angelle varieties show no alteration to the levels.

Determination of total antioxidant contents present in fruit harvested early and later in the season show a range of 0.4μM/mgDW (Dometica, R38) to 1.0μM/mgDW (Piccolo, R4) and 1.0μM/mgDW (Angelle, R26) to 1.6μM/mgDW (Island Beauty, R36 and Orange Baby Plum R35). In general there was a trend for higher antioxidant activity in the fruit from the later harvest compared to the fruit harvested early in the season. For example over a 2–fold increase that was statistically significant ($p < 0.05$) was determined in the Island Beauty and Dometica varieties over the two harvests. A comparison between locations (site A and B) for the Piccolo and Angelle varieties show no significant change in vitamin C levels or TEAC activities

Discussion

The data generated suggest that the timing of harvest early or late in the season can have an important effect on carotenoid and tocopherol content in the fruit. The fruit harvested later in the season possessed increased carotenoid (more precisely lycopene) and tocopherol contents. It is not surprising that lycopene and α-tocopherol were predominantly increased as these are the end-points of the biosynthetic pathways. Presumably, these data arise because during the early season the plants are still growing with vigour and energy is being utilised to create and maintain vegetative tissues. As the season progresses vegetative growth is reduced and the plant re-programmes its resources to fruit production. The higher light incidence may also contribute to increases in lycopene and tocopherols later in the season.

Perhaps the higher light incidence associated with production site B could have contributed to the higher lycopene and tocopherols found in some of the varieties included in the comparison of production sites.

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The differences associated with phenolic contents did not appear to be linked directly to the early and late timings of harvest but more with varietal differences, the exception being rutin which did appear to have an environmental effect surpassing genetic determinants. Interestingly, rutin is present in the peel of tomato fruit and acts as a protectant from light incidence. The production location of the fruit appeared to have no effect on the majority of phenolics tested. However chalcone-naringenin was unique to fruit derived from the more southerly site of the two compared. Again this compound is localised in the peel tissue of the fruit and acts as a protectant against light incidence. In conclusion if a grower wanted to generate a high flavonoid fruit the core factor would be the choice of variety.

Vitamin C content in fruit was not affected by harvesting early or late in season and the location of production site had no effect. This suggests that in the case of vitamin C the genetic effects are greater than the environment. Although not as clear as the lycopene and tocopherol profiles, the total antioxidant capacity of the fruit, as measured by the TEAC assays was greater in the later season fruit. Considering the increases in lycopene and tocopherol fruit content later in season perhaps it is not surprising that there is a general increase in TEAC activity with these fruit later in the season. Overall the production location did not affect the TEAC levels.

Conclusions

- The timing of harvests through the production season can have a profound effect on lycopene and tocopherol content in the fruit. The data suggest increased levels are found in fruit harvested later in the season. To a lesser extent these findings correlated and corroborated the total antioxidant activities determined.
- Phenolics and vitamin C showed no strong influence from the environmental effects of harvesting in the season.
- Site B did appear to enhance the content of some bioactive components but further studies are recommended.

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

Piccolo cultivated at site B both conventionally and organically showed no significant difference in carotenoid or tocopherol contents with both early and late season fruit (**Table 9**). Likewise fruit from the Angelle variety cultivated organically and conventionally at site B showed no difference in carotenoid or tocopherol contents (**Table 9**). Comparison between carotenoid and tocopherols in the Green Tiger variety indicated that the lycopene content was higher in the organically cultivated samples. Tocopherol content was reduced in the organic samples from Site B during the early season but increased in the late season. However, caution is required as no common production site was included for the Green Tiger variety. Therefore the difference could be due to location, or possibly in the stage of ripeness at analysis, not the mode of cultivation.

Piccolo fruit cultivated at site B early and later in the season showed no significant difference in phenolic content when cultivated conventionally or organically (**Table 10**), the only exception being chalcone-naringenin which was higher in conventional fruit samples. The same findings were determined with the Angelle variety cultivated at site A, again chalcone–naringenin being increased in the conventional fruit samples. The data suggested no difference in phenolic content of the Green Tiger variety cultivated by conventional or organic practice.

Vitamin C content and total antioxidant activity of the fruit from Piccolo, Angelle and Green Tiger cultivated by conventional and organic practice showed no significant differences (**Table 11**).

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Table 9. Carotenoid and tocopherol contents determined in ripe tomato fruit cultivated by organic and conventional practice. The representation and statistical treatment of the data is provided in the methods section. A= production site A and B = production site B. A[1] indicates site A was responsible for conventional[1] production and B[2] = organic[2] production.

Variety	UK source & Picking season	Cultivation mode	RHUL ID	Carotenoids and tocopherols (µg/gDW)					
				Phytoene	Phytofluene	Lycopene	ß-Carotene	Lutein	α-tocopherol
Piccolo	B-Early	Conventional	R2	199 ± 13	181 ± 6	303 ± 57	188 ± 6	171 ± 1	20 ± 4
		Organic	R3	198 ± 6	184 ± 3	355 ± 39	191 ± 8	170 ± 1	20 ± 2
Piccolo	B-Late	Conventional	R22	219 ± 4	205 ± 2	460 ± 11	208 ± 2	173 ± 0	31 ± 4
		Organic	R23	207 ± 2	199 ± 2	400 ± 14	215 ± 3	173 ± 1	34 ± 4
Angelle	B-Early	Conventional	R8	194 ± 15	180 ± 9	266 ± 84	180 ± 4	170 ± 1	8 ± 4
		Organic	R9	194 ± 11	183 ± 5	225 ± 47	176 ± 5	169 ± 1	8 ± 3
Angelle	B-Late	Conventional	R27	207 ± 5	194 ± 3	451 ± 14	199 ± 5	173 ± 0	20 ± 3
		Organic	R28	202 ± 3	193 ± 3	404 ± 33	200 ± 5	174 ± 1	22 ± 4
Green Tiger	A[1]/B[2]	Conventional[1]	R12	198 ± 22	186 ± 9	196 ± 18	183 ± 14	185 ± 18	25 ± 9
		Organic[2]	R13	187 ± 8	176 ± 4	439 ± 81	185 ± 5	174 ± 2	8 ± 5
Green Tiger	A[1]/B[2]	Conventional[1]	R31	229 ± 6	210 ± 3	505 ± 27	212 ± 6	181 ± 0	31 ± 5
		Organic[2]	R32	248 ± 6	245 ± 5	731 ± 25	261 ± 4	182 ± 1	50 ± 6

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Table 10. Phenolic contents determined in ripe tomato fruit cultivated by organic and conventional practice. The representation and statistical treatment of the data is provided in the methods section. A = production site A and B = -production site B. A^1 indicates site A was responsible for conventional[1] production and B^2 = organic[2] production.

Variety	UK source & Picking season	Cultivation mode	RHUL ID	Phenolics (mg/gDW)			
				Chlorogenic acid	Rutin	Naringenin	Chalcone Naringenin
Piccolo	B-Early	Conventional	R2	27 ± 2	6 ± 1	15 ± 3	21 ± 1
		Organic	R3	30 ± 4	10 ± 1	20 ± 2	1 ± 0.4
Piccolo	B-late	Conventional	R22	16 ± 0	5 ± 1	8 ± 3	5 ± 0
		Organic	R23	20 ± 1	5 ± 1	10 ± 5	4 ± 2
Angelle	B-Early	Conventional	R8	49 ± 12	4 ± 1	13 ± 6	6 ± 0
		Organic	R9	44 ± 5	5 ± 2	18 ± 2	1 ± 0.2
Angelle	B-Late	Conventional	R27	24 ± 1	8 ± 2	4 ± 2	5 ± 2
		Organic	R28	24 ± 1	8 ± 1	7 ± 6	9 ± 4
Green Tiger	A^1/B^{2-} Early	Conventional[1]	R12	10 ± 3	1 ± 0	15 ± 1	0
		Organic[2]	R13	16 ± 4	1 ± 0.1	19 ± 2	0
Green Tiger	A^1/B^{2-} Late	Conventional[1]	R31	12 ± 2	2 ± 0	12 ± 5	1 ± 0
		Organic[2]	R32	13 ± 2	3 ± 1	12 ± 4	4 ± 1

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Table 11. Vitamin C content and total antioxidant activity determined in ripe tomato fruit cultivated by organic and conventional practice. The representation and statistical treatment of the data is provided in the methods section. A = production site A and B = production site B. A^1 – indicates A was responsible for conventional[1] production and B^2 = organic[2] production.

Variety	UK source & Picking season	Cultivation mode	RHU L ID		
				Vitamin C mgVitC/100gDW	TEAC μM/mgDW
Piccolo	B-Early	Conventional	R2	74 ± 23	0.6 ± 0.1
		Organic	R3	60 ± 22	0.8 ± 0.1
Piccolo	B-late	Conventional	R22	34 ± 2	1.3 ± 0.1
		Organic	R23	44 ± 5	1.4 ± 0.1
Angelle	B-Early	Conventional	R8	38 ± 12	0.7 ± 0.1
		Organic	R9	37 ± 15	0.5 ± 0.1
Angelle	B-Late	Conventional	R27	29 ± 4	1.4 ± 0.1
		Organic	R28	20 ± 4	1.4 ± 0.2
Green Tiger	A^1/B^{2-} Early	Conventional[1]	R12	41 ± 10	0.7 ± 0.1
		Organic[2]	R13	59 ± 19	0.8 ± 0.1
Green Tiger	A^1/B^{2-} Late	Conventional[1]	R31	29 ± 4	1.1 ± 0.1
		Organic[2]	R32	29 ± 6	1.4 ± 0.1

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Discussion

In the present study the comparison between conventional and organic cultivated tomato fruit indicated that there was no effect on the bioactive content of the fruit. The only variation arose in the lycopene level of the organically cultivated Green Tiger variety. Unfortunately, it was difficult to ensure this finding was unambiguous, as the samples originated from two different production sites. In addition error could have arisen from the intrinsic green phenotype of this variety complicating sampling at the red ripe (stage 5) fruit phenotype.

Conclusions

- Conventional and organic cultivation practice had no impact on the bioactive content found in tomato fruit analysed in this study.

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

Piccolo, Angelle and Elegance sourced from Spain were compared to their UK counterparts cultivated at site A. The data generated for the carotenoid and tocopherol contents are provided in **Table 12**. In general Piccolo grown in Spain or the UK shows no significant change in their phytoene, phytofluene, β-carotene, and lutein or tocopherol fruit content, although some P values have shown significant increases in values predominantly from UK produced material. The lycopene content was elevated in the UK source Piccolo variety to a level whereby statistical analysis (student t-tests) suggest a significant ($p < 0.05$) increase in levels. A similar situation was observed with the Angelle variety with lycopene content in the UK fruit being increased, although the level of significance was calculated at $P < 0.001$. However, tocopherol content was reduced ($P<0.001$) by half in the UK fruit compared to their Spanish sourced counterparts. The Elegance variety contained similar amounts of phytoene, phytofluene, β-carotene and lutein between the Spanish and UK sourced samples, although in some cases the P values showed significant differences in favour of increased levels in UK generated fruit. Lycopene content in the UK fruit was increased by 2-fold ($P<0.01$), but tocopherol content was reduced by over 2-fold ($P<0.01$) compared to the Spanish sourced Elegance samples.

Comparison of the Elegance variety sourced from Holland with its UK comparator indicated small increases in phytoene, phytofluene, β-carotene, or tocopherol content. Lycopene content was increased and shown to be statistically significant ($P<0.01$) in the fruit source from Holland.

Comparison of the phenolic contents found in Spanish, Dutch and UK sourced samples indicated greater variability in levels (**Table 13**). The exception being Piccolo which when sourced from Spain showed no change in the majority of its phenolics measured. Angelle sourced from Spain contained increased chlorogenic acid ($P<0.001$), rutin and naringenin, the chlorogenic acid and rutin contents being over 2-fold higher than their UK counterparts. Elegance from Spain contained nearly double the chlorogenic acid content compared to the UK comparator with a significance of $P<0.05$, the presence of Chalcone naringenin was also unique (within the detection criteria used) to the Spanish sourced Elegance variety. Comparison between Dutch and UK source samples from the Elegance variety indicated that the UK samples contained twice as much chlorogenic acid and naringenin, while chalcone naringenin was elevated five–fold.

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Vitamin C and total antioxidant activities were determined in the Spanish, Dutch and UK sourced samples (**Table 14**). No significant changes in levels were found between samples.

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Table 12. Carotenoid and tocopherol contents determined in ripe tomato fruit sourced from Spain, UK and Holland. The representation and statistical treatment of the data is provided in the methods section. A = production site A and B = production site B. #[1] = mean of samples grown on two growth media rockwool and coir used at site A.

Variety	Collection point season & cultivation mode	Country of origin	RHUL ID	Carotenoids and tocopherol (μg/gDW)					
				Phytoene	Phytofluene	Lycopene	ß-Carotene	Lutein	α-tocopherol
Piccolo	A-Early conventional	Spain	R2	181 ± 7	174 ± 4	241± 44	205 ± 15	171 ± 1.2	22± 9
		UK#	R4	199 ± 13	181 ± 6	304 ± 57	188 ± 6	171 ± 1.2	20 ± 4
Angelle	A-Early conventional	Spain	R6	185 ± 5	180 ± 7	268 ± 48	190 ± 8	170 ± 1	18 ± 3
		UK#	R7	191 ± 4	180 ± 2	323 ± 41	182 ± 4	170 ± 1	9 ± 7
Elegance	A-Early conventional	Spain	R10	223 ± 25	196 ± 12	197 ± 12	187 ± 5	170 ± 2	36 ± 8
		UK#	R11	218 ± 12	186 ± 6	408 ± 98	181 ± 4	171 ± 1	13 ± 2
Elegance	A-Late Conventional	Holland	R30	246 ± 3	213 ± 1	506 ± 12	207 ± 2	171 ± 0.1	31 ± 4
		UK#	R29	234 ± 5	205 ± 3	423 ± 15	198 ± 5	174 ± 0.4	29 ± 3

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Table 13. Phenylpropanoid contents determined in ripe tomato fruit sourced from Spain, UK and Holland. The representation and statistical treatment of the data is provided in the methods section. A = material sourced from site A, # – material generated at site A.

Variety	Collection point season & cultivation mode	Country of origin	RHUL ID	Phenylpropanoids (μg/gDW)			
				Chlorogenic acid	Rutin	Naringenin	Chalcone Naringenin
Piccolo	A-Early Conventional	Spain	R2	28 ± 7	5 ± 3	16 ± 4	0
		UK#	R4	29 ± 3	6 ± 2	22 ± 4	0
Angelle	A-Early Conventional	Spain	R6	72 ± 7	11 ± 5	15 ± 1	0
		UK#	R7	35 ± 7	5 ± 2	13 ± 2	0
Elegance	A-Early Conventional	Spain	R10	52 ± 2	1 ± 1	6 ± 0	4
		UK#	R11	27 ± 1	0.3 ± 0.2	5 ± 3	0
Elegance	A-Late Conventional	Holland	R30	7 ± 2	3 ± 1	11 ± 4	0.3 ± 0.1
		UK#	R29	16 ± 3	2 ± 1	20 ± 4	1.2 ± 0.4

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Table 14. Vitamin C and TEAC contents determined in ripe tomato fruit sourced from Spain, UK and Holland. The representation and statistical treatment of the data is provided in the methods section. A = material sourced from site A, # – material generated at site A.

Variety	Collection point season & cultivation mode	Country of origin	RHUL ID	Vitamin C mgVitC/100 gDW	TEAC μM/mgDW
Piccolo	A-Early conventional	Spain	R2	51± 9	0.8 ± 0.1
		UK#	R4	59 ± 17	0.6 ± 0.1
Angelle	A-Early conventional	Spain	R6	33 ± 5	0.8 ± 0.1
		UK#	R7	32 ± 13	0.9 ± 0.1
Elegance	A-Early conventional	Spain	R10	19 ± 7	0.7 ± 0.3
		UK#	R11	17 ± 8	0.8 ± 0.1
Elegance	A-Late Conventional	Holland	R30	43 ± 3	0.6 ± 0.2
		UK#	R29	39 ± 4	0.8 ± 0.1

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Discussion

Within the sample set studied there is a trend towards an increased content of lycopene, associated with ripe fruit from UK sourced tomato varieties compared with fruit sourced from Spain. The opposite is true of the Dutch sourced samples which showed increased lycopene compared to the UK equivalents, although only one sample date (late season) was compared. In the case of tocopherol content the trend was clearer and suggested that the Spanish derived fruit contained more tocopherol. The Angelle and Elegance varieties sourced from Spain contained more phenolic compounds (up to 2 fold). Interestingly, given the intense colour of flavonoids and that they predominate in the peel of tomato fruit, the relative increases in compounds such as rutin and chalcone-naringenin, means it is feasible that the colour of the fruit sourced from Spain maybe effected, creating a more orange/yellow phenotype compared to the typical red colour. A comparable change in appearance between the Dutch and UK sources of the Elegance variety could also be predicted from the altered flavonoid content.

Despite these changes in carotenoid and phenolic contents the total antioxidant content did not change. There would appear to be a degree of balancing between antioxidant compounds. For example when more lycopene occurs less phenolics are found. This pattern could contribute to the overall lack of variation in total antioxidant content.

The use of identical varieties suggest that the changes arising are due to a combination of effects such as sampling, growth conditions in Spain and Holland and transportation through the supply chain.

Conclusions

- Analysis of the fruit sample set provided suggests the country of origin and/or supply chain to the UK can alter the content of bioactives.
- In general the data infer lycopene content could be lower in Spanish generated fruit but phenolic and tocopherol content higher. Overall the total antioxidant capacity of the fruit appears unaffected by the country of origin

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

Dometica is a classic supermarket variety, familiar to the consumer. Within the remit of Expt. 4, this variety was compared to Jack Hawkins which has been documented as a high lycopene variety previously; Pink Beef, and Island Beauty; 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.

As described in Expt. 1, fruit analysed from later in the season contained a higher lycopene content. This trend was consistent with the novelty varieties and the traditional varieties included in the sample set. Green Tiger was supplied from site A while all other varieties were from site B. This fact makes direct comparison of quantitative data difficult. It was clear that despite its green phenotype the carotenoid profile was similar to the traditional red varieties (**Table 15**). Taking into account the timing of harvest, phytoene levels were similar among all varieties apart from Orange Baby Plum, which had a higher content of phytoene and in comparison to most of the varieties had a higher phytoene to coloured carotenoid ratio. The fruit content of phytofluene, β-carotene and lutein in the varieties tested in Expt 1 were similar. In the case of lycopene content in ripe fruit, Island Beauty and Pink Beef contained more ($P<0.05$) lycopene compared to Dometica in the early season fruit. Late season fruit from the Green Tiger, Jack Hawkins and Island Beauty contained more lycopene ($P<0.05$) compared to the traditional Dometica variety (**Table 15**). It is also worth noting that the lycopene in Orange Baby Plum is pro-lycopene, not the common all-*trans* lycopene.

The tocopherol contents of all varieties were within the range of contents detected in the classic Dometica variety, the only exception being Orange Baby Plum where tocopherol levels were lower (**Table 15**).

The qualitative and quantitative differences associated with early and late season sampling make clear trends difficult to define. However, Orange Baby Plum is a high chlorogenic acid (phenylpropanoid) variety compared to Dometica (**Table 16**). This chemotype is independent of the sampling periods. Pink Beef and Island Beauty have comparatively high naringenin and chalcone–naringenin compared to Dometica varieties but only at the late stage of harvesting (**Table 16**). Of note also are the profiles generated from the Jack Hawkins and Pink Beef varieties. Here, during the early season, they give an appearance of a mutant defective in flavonoid biosynthesis but can be overcome in late season.

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All vitamin C fruit contents within the novelty varieties are within the range of the traditional Dometica variety when compared in the early season. Analysis of the late season solely suggests that the Pink Beef, Jack Hawkins and Island Beauty varieties are potentially high vitamin C containing tomatoes compared to Dometica (**Table 17**).

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 (**Table 17**).

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Table 15. Carotenoid and tocopherol contents determined in ripe tomato fruit collected in early or late season from novelty and traditional varieties. The representation and statistical treatment of the data is provided in the methods section. All samples were taken from conventional growing systems. A = production site A and B = production site B. #[2] = the lycopene present was prolycopene not all-trans lycopene.

Variety	Mode of cultivation & UK source	Picking season	RHUL ID	Carotenoids and tocopherols (µg/gDW)					
				Phytoene	Phytofluene	Lycopene	ß-Carotene	Lutein	α-tocopherol
Green Tiger	A	Early	R12	198 ± 22	186± 9	196 ± 18	183 ± 14	185 ± 18	25 ± 9
		Late	R31	229 ± 6	210 ± 3	505 ± 27	212 ± 6	181 ± 0	31 ± 5
Pink Beef	B	Early	R14	230 ± 24	188± 10	372 ± 78	188 ± 7	171 ± 1	17 ± 7
		Late	R33	226 ± 4	198 ± 3	417 ± 35	197 ± 4	171 ± 0	14 ± 4
Jack Hawkins	B	Early	R15	193 ± 7	175± 3	273 ± 49	175 ± 3	171 ± 1	11 ± 4
		Late	R34	232 ± 3	208 ± 3	548 ± 21	193 ± 2	176 ± 0	34 ± 6
Orange Baby Plum	B	Early	R16	273 ± 28	216 ± 14	250 ± 11 #[2]	172 ± 11	169 ± 1	6 ± 0
		Late	R35	277 ± 7	230 ± 4	390 ± 18 #[2]	187 ± 2	173 ± 0	19 ± 3
Island Beauty	B	Early	R17	208 ± 13	198 ±11	442 ± 124	196 ± 12	170 ± 1	24 ± 14
		Late	R36	233 ± 4	211 ± 3	573 ± 43	224 ± 5	174 ± 0	37 ± 3
Super Sweetini	A	Early	R18	200 ± 23	175 ± 22	188 ± 16	188 ± 4	174 ± 2	24 ± 5
		Late	R37	229 ± 3	205 ± 2	403 ± 17	221 ± 5	176 ± 0	37 ± 3
Dometica	B	Early	R20	180 ± 7	180± 7	232 ± 69	188 ± 13	171 ± 13	15 ± 1
		Late	R38	218 ± 4	202 ± 3	487 ± 27	207 ± 5	174 ± 1	31 ± 7

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Table 16. Phenylpropanoids contents determined in ripe tomato fruit collected in early or late season from novelty and traditional varieties. The representation and statistical treatment of the data is provided in the methods section. All samples were taken from conventional growing systems. A = production site A and B = production site B. #[2] = the lycopene present was prolycopene not all-trans lycopene.

Variety	UK source	Picking season	RHUL ID	Phenylpropanoids (μg/gDW)			
				Chlorogenic acid	Rutin	Naringenin	Chalcone Naringenin
Green Tiger	A	Early	R12	10 ± 3	1 ± 0	15 ± 1	0
		Late	R31	12 ± 2	2 ± 0	12 ± 5	1 ± 0
Pink Beef	B	Early	R14	15 ± 0	0	3 ± 0	0
		Late	R33	20 ± 4	1 ± 0	31 ± 11	0
Jack Hawkins	B	Early	R15	15 ± 0	0	2 ± 0	0
		Late	R34	15 ± 4	2 ± 2	18 ± 6	4 ± 3
Orange Baby Plum	B	Early	R16	71 ± 13	7 ± 3	8 ± 4	1 ± 0
		Late	R35	50 ± 2	9 ± 1	0	12 ± 3
Island Beauty	B	Early	R17	5 ± 1	1 ± 0	7 ± 1	0
		Late	R36	18 ± 1	3 ± 1	23 ± 4	6 ± 4
Super Sweetini	A	Early	R18	13 ± 2	11 ± 0	18 ± 3	0
		Late	R37	16 ± 3	13 ± 0	16 ± 1	2 ± 1
Dometica	B	Early	R20	5 ± 1	1 ± 0	8 ± 2	0
		Late	R38	15 ± 1	2 ± 1	12 ± 3	1 ± 1

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Table 17. Vitamin C and total antioxidant activity (TEAC) contents determined in ripe tomato fruit collected in early or late season from novelty and traditional varieties. The representation and statistical treatment of the data is provided in the methods section. All samples were taken from conventional growing systems. A = production site A and B = production site B.

Variety	UK source	Picking season	RHUL ID		
				Vitamin C mgVitC/100gDW	TEAC μM/mgDW
Green Tiger	A	Early	R12	41 ± 10	0.7 ± 0.1
		Late	R31	29 ± 4	1.1 ± 0.1
Pink Beef	B	Early	R14	36 ± 10	0.7 ± 0.1
		Late	R33	63 ± 4	1.2 ± 0.2
Jack Hawkins	B	Early	R15	37 ± 18	0.8 ± 0.3
		Late	R34	57 ± 6	1.2 ± 0.1
Orange Baby Plum	B	Early	R16	75 ± 20	0.8 ± 0.07
		Late	R35	31 ± 6	1.6 ± 0.1
Island Beauty	B	Early	R17	25 ± 7	0.5 ± 0.1
		Late	R36	42 ± 8	2 ± 0.2
Super Sweetini	A	Early	R18	35 ± 6	0.7 ± 0.2
		Late	R37	31 ± 6	1.2 ± 0.1
Dometica	B	Early	R20	74 ± 29	0.4 ± 0.1
		Late	R38	28 ± 5	1.3 ± 0.1

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Discussion

The novelty varieties Green Tiger, Jack Hawkins and Island Beauty are potentially high lycopene containing varieties. However, this potential over the traditional variety Dometica appears to only hold true in the harvests later in the season. Compositionally all varieties were similar with the exception of Orange Baby Plum. Here the high phytoene is a classic profile of the *CRTISO* allele. Mutations in this gene block/reduce carotene isomerisation preventing the conversion of prolycopene to all-*trans* lycopene which impacts on cyclisation (**Figure 1**). The presence of prolycopene is important as it is this form of lycopene that is believed to be more bioavailable. In addition phytoene is becoming an important ingredient in cosmetics and new renewable sources are a priority presently.

Environmental effects appear to influence the phenylpropanoid/flavonoid content dramatically. Collectively the novelty varieties do not appear to have elevated phenylpropanoids or flavonoids, compared to Super Sweetini or Dometica varieties. The exception is the Orange Baby Plum varieties which could be used as a high phenylpropanoid variety. There appears to be a clear divide between varieties, when the data from Expt. 1, is included. This complete data set suggests Piccolo, Angelle and Orange Baby Plum are varieties high in phenylpropanoids, while the other lines accumulate flavonoids. These findings are independent of the environmental conditions and a genetic determinant could be involved. Overall the novelty varieties seem to be more susceptible to environmental/harvesting regimes.

Conclusions

- The novelty varieties do have important differences in their qualitative and quantitative bioactive profiles.
- Potential exists to deliver different and higher bioactives using these varieties.
- The environmental seasonal effects require further investigation in these varieties.
- Assessment of fruit ripeness in non-red varieties or those with patterned colour present particular difficulties.

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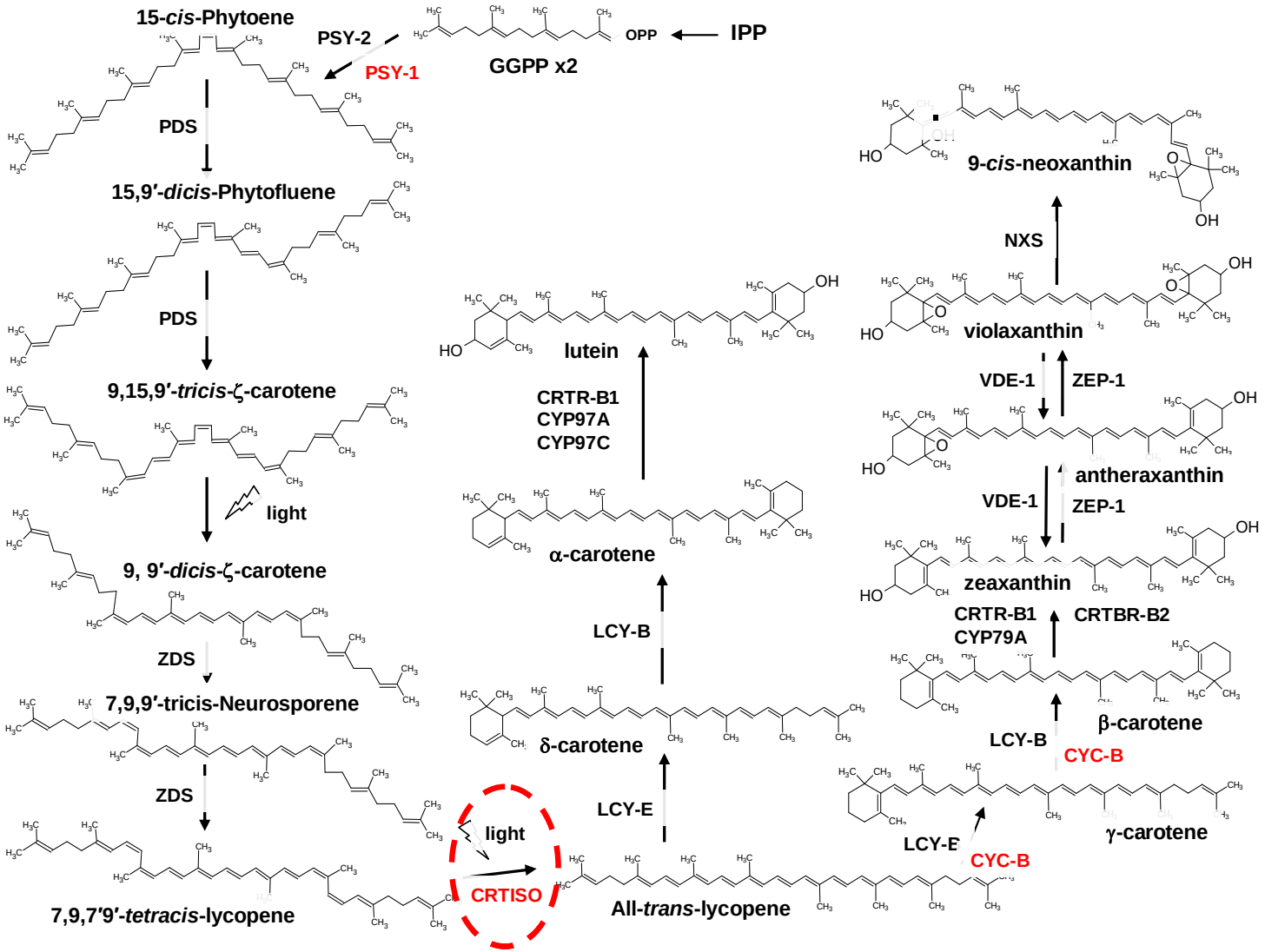

Figure 1. Carotenoid biosynthesis in higher plants. The reaction catalysed by CRTISO is marked with a dashed red line/circle. This is predicted to be the candidate allele in the Orange Baby Plum variety and responsible for the pro-lycopene phenotype.

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

The *RIN* varieties used in this study were sourced and delivered to Royal Holloway after the duration of the main project. 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. Despite these logistical problems, analysis has been performed but it must be taken into account that concurrent analysis with the other varieties was not performed.

The phytoene, phytofluene, β-carotene and lutein contents were comparable to those determined in late season for the other varieties described in Expt 1 and 4 (**Table 18**). Lycopene and tocopherol content in the fruit was higher than the varieties with the highest amounts of lycopene and tocopherol determined in Expt 1 and 4 (tables 6 & 15). These findings were particularly impressive in the Ninette variety, which at 873μg/gDW lycopene was approximately 300μg/gDW better than the Island Beauty variety which contained the highest content in the previous Expts. The tocopherol level also represented a 2-fold increase in this variety compared to the varietal ranges determined in Expt 1 and 4.

In contrast phenolic contents (phenypropanoids and flavonoids) were lower in these *RIN* varieties, in some cases an order of magnitude less compared to contents ascertained with varieties analysed in Expt 1 and 4 harvested later in the season (**Table 19**). The vitamin C and total antioxidant activities were similar between the two varieties analysed and within the range of contents/activity determined in Expt. 1 and 4 (**Table 20**).

Discussion

The *RIN* Varieties analysed appeared to contain increased lycopene contents. One explanation could be that the longer shelf-life facilitates a longer period of synthesis in which lycopene will accumulate, even during the supply chain. In contrast to the carotenoids the phenylpropanoids appear to be lower in content. Therefore potentially the effects of the *RIN* background are greater on phenylpropanoid content than carotenoids. Based on this small sample set that was not analysed concurrently with the varieties provided for Expt1 and 4 it would appear the *RIN* background cultivated in Spain and subjected to the supply chain has limited, perhaps even improved effect on bioactive contents.

Conclusions

- Based on this small sample set that was not analysed concurrently with the varieties provided for Expt 1 and 4, it would appear the RIN background cultivated in Spain

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and subjected to the supply chain has limited or even improved effect on the type and amounts of bioactives present in the fruit.

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Table 18. Carotenoid and tocopherol contents found ripe fruit from in *RIN* varieties . The representation and statistical treatment of the data is provided in the methods section. Material was sourced from Spain and distributed via production site A. Justyna and Ninette are the *RIN* varieties analysed.

Variety	Mode of cultivation & source	Delivery	RHUL ID	Carotenoids and tocopherols (µg/gDW)					
				Phytoene	Phytofluene	Lycopene	ß-Carotene	Lutein	α-tocopherol
Justyna	Conventional Spain	Dec	R39	257 ± 15	225 ± 10	565 ± 88	211 ± 6	172 ± 2	31 ± 3
Ninette	Conventional Spain	Dec	R40	328 ± 40	269 ± 27	873 ± 114	230 ± 10	175 ± 1	66 ± 12

Table 19. Phenylpropanoid contents found in ripe fruit from *RIN* varieties. The representation and statistical treatment of the data is provided in the methods section. Material was sourced from Spain and distributed via the production site A. Justyna and Ninette are the *RIN* varieties analysed.

Variety	Mode of cultivation & source	Delivery	RHUL ID	Phenylpropanoids (µg/gDW)			
				Chlorogenic acid	Rutin	Naringenin	Chalcone Naringenin
Justyna	Conventional Spain	Dec	R39	4 ± 1	1 ± 0.2	0	3 ± 1
Ninette	Conventional Spain	Dec	R40	3 ± 1	2 ± 1	0	3 ± 2

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Table 20. Vitamin C and Total antioxidant activity (TEAC) found in ripe fruit from *RIN* varieties. The representation and statistical treatment of the data is provided in the methods section. Material was sourced from Spain and distributed via production site A. Justyna and Ninette are the *RIN* varieties analysed.

Variety	Mode of cultivation & source	Delivery	RHUL ID		
				Vitamin C mgVitC/100 gDW	TEAC μM/mgDW
Justyna	Conventional Spain	Dec	R39	26 ± 4	1 ± 0.1
NInette	Conventional Spain	Dec	R40	29 ± 7	1.3 ± 0.2

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Comparison of bioactive levels found in TOMCOM study compared to reference levels

The methods used in the present study 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 (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 the Vitamin A Recommended Daily allowance (RDA), 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.

Table 21. Comparison of bioactive contents determined in the TOMCOM study with reference data.
[1]MacCance and Widdowson's, 2004, The composition of Foods: Summary Ed 6th, Cambridge: Royal Society of Chemistry)
[2]USDA National Nutrient Database for Standard Reference, Release 20.
[3] Early season, [4] Late season

Bioactive	**Reference levels [1&2]**		**Present study**	
	mg/gFW	RDA (%) per gFW	mg/gFW	RDA (%) per gFW
Lycopene	26[1] 30[2]	- -	33 – 70[4] 20 – 40[3]	- -
b-carotene	4.5[1&2]	2.3 (IU), (0.3%)	18 - 22	5.0 (IU), (0.6%)
Lutein	1.1[2]	-	20.0	
Vit E	5.0[2]	0.03%	3 - 5	0.03%
Vit C	130 [1&2]	0.2%	20 - 100	0.03 – 0.16

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Annex 1. Fruit parameters for the samples analysed

Variety	Average %DM	SD
Small 50 -80g		
Piccolo	12.4	1.1
Angelle	12.8	1.2
Orange Baby Plum	10.0	0.9
Medium (160 to 200g)		
Green Tiger	9.1	0.9
Amoroso	7.8	0.8
Super Sweetini	8.3	0.9
Ninette	8.9	0.9
Justyna	8.6	0.9
Large (150- 450g)		
Jack Hawkins	6.7	1.8
Pink Beef	6.1	1.7
Elegance	7.1	0.7
Island Beauty	4.0	1.8
Dometica	6.6	1.8

This table contains the supplementary information to facilitate the calculation of bioactive content on a fresh weight 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. The dry matter figures represent the mean of 6 biological replicates and, given the uniformity of dry matter content from sample to sample within a variety, can be used to represent all samples of that variety. The dry matter content for Island Beauty is low compared with similar varieties but this figure has been rechecked and validated for the samples processed within this study.

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