Project title:	Extend the marketing period of Gala apples. Phase I: Establishing analytical methods to assess flavour.
Project number:	TF 213
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Report:	Final report, August 2014
Previous report:	N/A
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Location of project:	Produce Quality Centre, East Malling Research
Industry Representative:	Nigel Jenner, Norman Collett Ltd.
Date project commenced:	1 April 2013
Date project completed (or expected completion date):	31 July 2014

[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.]

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

- Gala storage in 5% CO_2 and 1% Oxygen (5/1) with or without SmartFresh^{TM} was better for extending the storage quality of fruits over fruits stored in 1% O_2 with or without SmartFresh^{TM}.
- Orchards producing Gala with high dry matter content (16 % DM) maintained their texture and overall taste panel acceptability for longer during extended storage.

Background and expected deliverables

UK production of Gala is expected to increase by 40% over the next four years. To provide a market for this fruit there is a need to extend the marketing period of UK Gala into April/ May. The challenge facing growers storing Gala beyond late March/early April is to compete with new season imports from the Southern hemisphere which are perceived to have superior quality at this stage of the season.

For Gala a number of storage regimes are currently being used; a regime of <1% CO_2, 1% O_2 at 0.5°C will allow storage up to February. Extending storage into March or early April requires the use of 5% CO_2, 1% O_2 at 1.5°C to prevent the development of scald and loss of firmness during shelf-life. There have been several reports indicating that after long-term controlled atmosphere (CA) storage, Gala apples have less flavour, relating to a decrease in flavour volatiles. Low oxygen concentrations and high carbon dioxide concentrations both contribute to this effect. Work funded by APRC (Stow and Genge, 2000) looking at storage in a range of CO_2 (0, 2.5 and 5% CO_2) and O_2 (1, 1.5 and 2%) conditions, reported that flavour production in general declined after 110 days of storage, and that where CO_2 was present in the atmosphere, flavour volatile production was suppressed. The highest flavour production was reported at the highest oxygen concentration tested (2%).

Given the advent of alternative strategies to control scald and reduce softening, such as SmartFresh^{TM}, ethylene scrubbing and ultra-low oxygen storage such as used with dynamic controlled atmosphere (DCA) technologies, the need to incorporate 5% CO_2 in the storage atmosphere may be less important for longer term storage. It is vital for UK growers to define the best storage regimes for long-term storage of Gala where flavour production can be maintained for longer.

There have been many studies to identify volatiles in terms of their contribution to apple flavour. Methods for volatile capture by Solid Phase Micro extraction (SPME), or Thermal

Desorption techniques followed by analysis using Gas Chromatograph with Mass spectroscopy (GC-MS), are currently under further investigation at NRI.

A number of studies have charted the fall in volatile production during CA and air storage, and, as mentioned above, it is well documented that CA storage suppresses production of flavour production with time. However, some studies (Stow and Genge, 2000; Plotto *et al.,* 1999) have found a poor correlation between ester flavour volatile decline and taste panel assessments of good eating quality, which suggests other attributes such as sweetness and acidity and texture (crispness) are also essential for the overall sensory perception of fruit quality. This underlines the importance of including the full range of characteristics in any assessment of eating quality of flavour

This project seeks to define a practical protocol for assessing Gala flavour in terms of volatiles and flesh composition that can be used to optimise pre and post-harvest practices to maintain flavour, as well as identifying key orchards with fruit suitable for long-term storage. Moreover, a preliminary study is being undertaken on the effects of picking date, storage regime and SmartFresh^{TM} application on flavour retention of long-term stored fruit.

In addition to HDC funds, co-funding from this project has been kindly provided by Norman Collett, World Wide Fruit, A.C. Goatham and Son, Chingford and Mansfields. Further funding by AgroFresh allowed an investigation of the role of SmartFresh^{TM} on eating quality of fruit stored under alternative CA regimes.

Summary of the project and main conclusions

Gala clones from 6 orchards (A-F) on two picking dates were used. The harvest quality is summarised in Tables 1 and 2.

Table 1 Harvest quality of Gala apples picked from six orchards on two picking dates. Each data point is the mean of measurements on two samples of 10 fruit.

Orchard	Pick 1			Pick 2		
	Firmness (N)	% Brix	CTIFL Starch score	Firmness (N)	% Brix	CTIFL Starch score
A	91.84	10.6	2.8	n.d.	11.4	3.6
B	93.10	10.4	1.6	92.00	11.6	4.6
C	89.30	10.4	1.6	90.76	11.6	4.7
D	103.02	12.0	1.4	98.42	11.8	3.9
E	98.35	10.8	1.4	97.08	12.1	4.0
F	84.67	11.4	3.4	n.d.	11.5	4.1

Table 2 Dry matter (%) content, respiration rate (ml CO_2/kg/h), internal ethylene concentration (ppb) of Gala sourced from 6 orchards over two picking dates

Orchard	Pick 1			Pick 2		
	% Dry Matter	Resp rate	I.E.C. (ppb)	% Dry Matter	Resp rate	I.E.C (ppb)
A	13.7	1.21	129.3	15.2	1.17	777.1
B	14.0	1.16	82.3	14.8	1.48	103.1
C	16.8	1.31	78.6	15.5	1.59	576.8
D	16.5	1.22	372.2	15.9	1.14	824.4
E	14.4	1.32	345.0	14.7	1.53	792.9
F	13.6	1.20	58.6	13.5	1.54	807.4

Apples from orchards with a higher dry matter content at harvest tended to have higher soluble solids content at harvest, which resulted in fruit with a higher % Brix (15.5-16.0%) during the storage season.

Fruit with a higher DM content produced fruit that had higher fruit firmness as measured by penetrometer and by sensory evaluation.

The combination of higher fruit firmness and high % Brix, had a large contribution towards raising the overall acceptability of Pick 1 fruit tasted in April, but the relationship weakened in fruit tasted in June and with later picked fruit.

The fruit was stored under a range of CA regimes (summarised in Tables 3 and 4) and assessed in April and in June.

The influence of CA regimes depended on the time of the year fruit was tasted.

Table 3 summarises the overall acceptability of fruit assessed in April after six months of storage. The assessments were carried out by experienced Gala growers/marketers.

In April, the two orchards where fruits were high in % Brix and dry matter content (C and D), scored particularly highly following storage in 5%CO_2, 1% O_2 (5/1) at 1.5°C and picked at 80% starch with no SF-treatment. Storage in 3% CO2 and 2% O2 led to the highest overall acceptability score but when compared to overall acceptability scores in some orchard consignments however, when averaged across all six orchards, there was no statistical difference in the 5%CO_2, 1% O_2 regime with the other regimes tested.

Comparison of storage treatments confirm earlier observations that storing fruit in the absence of CO_2 leads to poorer fruit texture compared to fruit stored in 5% CO2.

Firmness of fruit was higher when kept in 5/1 CA stored at 0.5°C (HDC 4) compared to fruit stored in 1.0% O_2 <1% CO_2 (HDC 7) In April the effect of SF on firmness was not observed in Pick 1 fruit (80% Starch) but improved firmness retention in later picked fruit (60% Starch).

Table 3 Overall acceptability of Gala apples in April following storage under a range of storage treatments followed by 3 days at 15°C and 2 days at ambient. Fruit were picked from 6 orchards on two picking dates. Each data point is the mean of assessments by 7 tasters.

PICK 1	Storage atmos	Temp	Harvest maturity (% starch)	SF?	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F	Mean
AG 1	3%CO_2, 2%O_2	0.5°C	80%	+SF	5.75	5.75	6.00	6.50	6.38	6.63	6.17
AG 2	1.5%CO_2, 1.5%O_2	0.5°C	80%	+SF	6.38	5.38	6.50	5.88	5.88	5.63	5.94
AG 3	<1%CO_2, 1.2 %O_2	0.5°C	80%	+SF	5.71	5.75	6.00	6.38	5.75	5.88	5.91
AG 4	3%CO_2, 2%O_2 with conditioning	0.5°C	80%	+SF	6.83	5.86	5.00	6.86	5.43	5.50	5.91
HDC 1	5%CO_2, 1%O_2	1.5°C	80%	+SF	5.57	5.13	6.25	6.25	6.00	5.00	5.70
HDC 2	5%CO_2, 1%O_2	1.5°C	80%	-SF	5.43	5.50	7.25	7.25	4.63	4.86	5.82
HDC 3	5%CO_2, 1%O_2	0.5°C	80%	+SF	6.00	5.63	5.63	5.44	6.00	6.31	5.83
HDC 4	5%CO_2, 1%O_2	0.5°C	80%	-SF	6.38	5.86	6.63	5.13	6.75	5.00	5.96
HDC 5	<1%CO_2, 1.0 %O_2	0.5°C	80%	+SF	5.00	4.13	6.14	5.75	6.06	5.25	5.39
HDC 7	<1%CO_2, 1.0 %O_2	0.5°C	80%	-SF	4.69	6.13	5.00	6.25	4.98	4.38	5.24
mean					**5.77**	**5.51**	**6.04**	**6.17**	**5.78**	**5.44**	

PICK 2					Orch A	Orch B	Orch C	Orch D	Orch E	Orch F	Mean
AG 1	3%CO_2, 2%O_2	0.5°C	60%	+SF	6	6.75	5.25	7	6.25	4.71	5.99
AG 2	1.5%CO_2, 1.5%O_2	0.5°C	60%	+SF	6	5.43	5.88	5.48	6.13	4.38	5.55
AG3	<1%CO_2, 1.2 %O_2	0.5°C	60%	+SF	6.25	5.13	4.43	6.75	5.88	5.68	5.68
AG4	3%CO_2, 2%O_2 with conditioning	0.5°C	60%	+SF	5.07	5.29	6.21	6.43	6.14	6.9	6.01
HDC 6	<1%CO_2, 1.0 %O_2	0.5°C	60%	+SF	6.04	6.38	5.75	4.88	6.25	5.75	5.84
HDC 8	<1%CO_2, 1.0 %O_2	0.5°C	60%	-SF	5.14	5	3.75	4.75	5.44	5.75	4.97
mean					**5.75**	**5.66**	**5.21**	**5.88**	**6.01**	**5.53**	

Table 4 Overall acceptability of Gala apples in June following storage under a range of storage treatments followed by 3 days at 15°C and 2 days at ambient. Fruit were picked from 6 orchards on two picking dates. Each data point is the mean of assessments by 7 tasters. An ANOVA was carried out for HDC and AG treatments separately.

	Storage atmosphere	Temp	Harvest maturity (% starch)	SF?	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F	mean
AG 1	3%CO_2, 2%O_2	0.5°C	80%	+SF	5.50	5.25	5.63	6.50	6.00	5.81	5.78
AG 2	1.5%CO_2, 1.5%O_2	0.5°C	80%	+SF	6.13	4.61	5.57	5.43	6.29	5.71	5.62
AG 3	<1%CO_2, 1.2 %O_2	0.5°C	80%	+SF	5.29	6.00	6.43	5.64	6.25	4.88	5.75
AG 4	3%CO_2, 2%O_2 with conditioning	0.5°C	80%	+SF	5.57	5.36	6.29	5.00	5.78	4.86	5.47
HDC 1	5%CO_2, 1%O_2	1.5°C	80%	+SF	5.56	5.79	5.71	5.38	6.00	6.25	5.78
HDC 2	5%CO_2, 1%O_2	1.5°C	80%	-SF	5.71	5.38	6.43	4.29	4.88	5.63	5.38
HDC 3	5%CO_2, 1%O_2	0.5°C	80%	+SF	5.83	5.75	5.94	6.22	5.71	6.81	6.04
HDC 4	5%CO_2, 1%O_2	0.5°C	80%	-SF	5.63	4.63	5.50	5.79	5.38	4.63	5.26
HDC 5	<1%CO_2, 1.0 %O_2	0.5°C	80%	+SF	6.00	5.63	5.69	5.71	5.06	6.00	5.68
HDC 7	<1%CO_2, 1.0 %O_2	0.5°C	80%	-SF	4.17	4.44	4.00	4.29	5.63	4.88	4.56
mean					**5.54**	**5.28**	**5.72**	**5.42**	**5.70**	**5.54**	
					Orch A	Orch B	Orch C	Orch D	Orch E	Orch F	**mean**
AG 1	3%CO_2, 2%O_2	0.5°C	60%	+SF	6.25	5.50	5.00	6.00	6.50	5.63	**5.81**
AG 2	1.5%CO_2, 1.5%O_2	0.5°C	60%	+SF	6.25	5.75	5.57	6.00	5.29	5.38	**5.71**
AG 3	<1%CO_2, 1.2 %O_2	0.5°C	60%	+SF	4.86	4.29	4.36	6.00	5.14	4.63	**4.88**
AG 4	3%CO_2, 2%O_2 with conditioning	0.5°C	60%	+SF	5.83	6.06	4.25	6.13	5.00	4.60	**5.31**
HDC 6	<1%CO_2, 1.0 %O_2	0.5°C	60%	+SF	5.86	6.50	5.29	6.31	5.71	4.44	**5.69**
HDC 8	<1%CO_2, 1.0 %O_2	0.5°C	60%	-SF	3.86	3.33	4.57	4.57	3.75	4.75	**4.14**
mean					**5.48**	**5.24**	**4.84**	**5.83**	**5.23**	**4.90**	

In June the overall acceptability scores of fruit had dropped slightly from the April assessment. Moreover, differences in overall acceptability between orchards were lower than in the April assessment. Averaged across all treatments, orchard C and E scored slightly better overall acceptability than the other orchards.

The highest scoring regime was 5/1 picked at 80% starch + SmartFresh^{TM}. However, in comparison with other 5/1 treatments, no significant effects between treatments were observed.

When averaged across all orchards the effect of modifying CO_2 or O_2 (AG-treatments) on overall acceptability was similar to storing fruit in 5/1. However, there was a strong orchard interaction with CA regimes and in orchards that had higher dry matter content 3% CO_2, 2% O_2 and 1.5% CO_2, 1.5% O_2 led to fruit with good overall acceptability. Interestingly storing Pick 1 fruit (80% starch) in 1% O_2 with SF provided fruit of equal acceptability,

No effect of storage temperature was seen on fruit firmness when Gala was stored in 5/1 (8.7-9.0 kg). Application of SF led to a small increase in firmness retention (9.2-9.3 kg) kg).In later Pick 2 fruit (60% starch) SF significantly improved firmness retention (8.8 kg) compared to untreated Gala (6.1 kg) stored in 1% O_2, <1% CO_2.

Fruit firmness of Gala stored until June was strongly influenced by the dry matter content of fruit. Orchards C, D, and F retained better fruit firmness during long-term storage. Picking fruit at 80% starch helped to retain fruit firmness.

Conclusions

- There was found to be a strong influence of orchard on the eating quality of long-term stored Gala.
- High dry matter content in Gala led to fruit with increased % Brix content and better firmness retention during storage.
- Fruit with higher % Brix and firmness were generally considered to have better overall acceptability.
- Untreated Gala at 85-80% starch background provided fruit with a better eating quality than later picked fruit in long-term storage.
- Later picked fruit (60 % starch) from some orchards treated with SmartFresh^{TM} were able to retain acceptable firmness and eating quality in April and June.
- Storing Gala in 5% CO_2, 1% O_2 either at 0.5 or 1.5°C, retained similar eating quality in April and June.

- Gala stored in 1% Oxygen with SmartFresh™ were in general of similar eating quality to 5/1 stored fruit.
- Alternative storage regime of 3% CO_2 and 1% O_2 and 1.5% CO_2 and 1.5% O_2 treated with SmartFresh™ provided fruit of good eating quality in April and June.

Financial benefits

The results presented in this report were obtained from a single season, and therefore need to be validated before financial benefits can be estimated.

Action points for growers

The results presented in this report were obtained from a single season, and therefore need to be validated before action points can be provided.

SCIENCE SECTION

Introduction

UK production of the apple cultivar Gala is expected to increase by 40% over the next four years. To provide a market for this fruit there is a need to extend the marketing period of UK Gala into April/ May. The challenge facing growers storing Gala beyond late March/early April is to compete with new season imports from the Southern hemisphere which are perceived to have superior quality at this stage of the season.

For Gala a number of storage regimes are currently being used; a regime of <1% CO_2, 1% O_2 at 0.5°C will allow storage up to February. Extending storage into March or early April requires the use of 5% CO_2, 1% O_2 at 1.5°C to prevent the development of scald and loss of firmness during shelf-life. There have been several reports that after long-term controlled atmosphere (CA) storage Gala apples have less flavour, relating to a decrease in flavour volatiles (Plotto *et al*. 2000, Stow and Genge, 2000). Low oxygen concentrations and high carbon dioxide concentrations both contribute to this effect. Work funded by APRC (Stow and Genge, 2000) looking at storage in a range of CO_2 (0, 2.5 and 5% CO_2) and O_2 (1, 1.5 and 2%) conditions reported that flavour production in general declined after 110 days of storage and that, where CO_2 was present in the atmosphere flavour volatile production was suppressed, the highest flavour production was reported in 2% oxygen.

With the advent of alternative strategies to reduce softening and control scald development (SmartFresh™, ethylene scrubbing, ultra-low oxygen storage such as used with dynamic controlled atmosphere (DCA) technologies) the need to incorporate 5% CO_2 in the storage atmosphere may be avoided, or at least reduced, for longer term storage. Nonetheless, the suppression of ethylene synthesis and use of low O_2/high CO_2 storage can reduce the synthesis of precursors of flavour volatile production. A re-definition of the best storage regimes for long-term storage of Gala, where flavour production can be maintained for longer, is vital for UK Gala apple growers.

There have been many studies to identify volatiles in terms of their contribution to apple flavour. Studies in the US and New Zealand have identified the major components of ‘fruity’ aroma/flavour attributes associated with apple. Hexyl acetate, butyl acetate and 2-methylbutyl acetate have been identified as the primary volatiles responsible for apple aroma in several cultivars, including Gala. Hexyl acetate was the volatile contributing most to apple ‘fruity’ and pear flavour and is a major component of the volatiles produced by Gala, but also one of the three most important in the perception of flavour by sensory panellists (Young *et al*. 1996; Plotto 1999). Methods for volatile capture by Solid Phase

Micro extraction (SPME), or Thermal Desorption techniques followed by analysis using Gas Chromatograph with Mass Spectroscopy (GC-MS) are currently under investigation at NRI.

A number of studies have charted the fall in volatile production during CA and air storage, and, as mentioned above, it is well documented that CA storage suppresses production of flavour production with time. Hexyl acetate production is suppressed by elevated CO_2 in the atmosphere but responds well to increasing concentration of O_2 (Stow and Genge, 2000), increasing O_2 from 1 to 2% doubled the amount of hexyl acetate. Other volatiles such as 2-methylbutyl acetate, methyl-2-methylbutyrate, ethyl 2 methylbutyrate and propyl-2-methylbutyrate are also associated with Gala flavour. These all appear to decline during storage. These ester aromas may be important for general fruity and sweet aromas.

However, some studies (Stow and Genge, 2000; Plotto *et al.,* 1999) have found a poor correlation between ester flavour volatile decline and taste panel assessments of good eating quality, which suggests that other attributes, such as sweetness and acidity and texture (crispness), are also essential for the overall sensory perception of fruit quality. This underlines the importance of including the full range of characteristics in any assessment of eating quality or flavour

This Phase 1 project seeks to define a practical protocol for assessing Gala flavour in terms of volatiles and flesh composition that can be used to optimise pre and postharvest practices to maintain flavour, as well as identifying key orchards with fruit suitable for long-term storage. Moreover, a preliminary effect of picking date and SmartFresh™ application on flavour development during 5% CO_2 1% O_2 CA storage will provide initial information on the effect of fruit maturity on the eating quality of long-term stored fruit.

In a future project (Phase 2) it is anticipated that the orchards selected as producing good fruit will be used to allow direct comparison of standard Gala CA storage regimes (5% CO_2, 1% O_2 at 1.5°C) and alternative CA regimes (based on lower CO_2 higher O_2 with or without SmartFresh™). Orchard practices (tree age, rootstock, thinning, pruning, fertigation) of orchards that yield the best eating quality will be used to identify best practice in orchard management and harvesting and storage techniques for the industry to adopt.

Co-funding from this project has been kindly provided by Norman Collett, World Wide Fruit, A.C. Goatham and Son, Chingford and Mansfields. Further funding provided by AgroFresh allowed the investigation to be expanded to study the effect of SmartFresh™ on eating quality of fruit stored under alternative CA regimes.

Materials and methods

Gala fruit was supplied from six orchards on two picking dates (24 September and 2 October 2013) chosen to correspond to 80% starch (Pick 1) and 60% starch (Pick 2). The picking date was decided on the basis of information provided through the Quality Fruit Group on the progress of ripening in the 2013 season.

On arrival at the Produce Quality Centre storage facility (Jim Mount Building, EMR) the fruit was randomised and divided among the storage treatments which are shown in Table 5. The fruit for each storage treatment was stored within a 360 L controlled atmosphere (CA) chamber able to hold six 15 Kg boxes. The treatments are referred to as "HDC" treatments and "AG" treatments. "HDC" treatments were funded through HDC, Norman Collett, World Wide Fruit, A.C. Goatham and Son, Chingford and Mansfields. "AG" treatments were funded by Agrofresh. Each HDC treatment chamber contained one box of each of the six orchards. Each AG treatment chamber included samples from two picks from each of the six orchards. These twelve samples were placed as nets within the CA chambers.

Where appropriate, SmartFresh^{TM} treatment (625 ppb 24 hours) was initiated within 10 hours of fruit arrival at the facility.

CA was established immediately or after SmartFresh^{TM} treatment. The only exception was that the first pick fruit for AG treatments was stored in air until the second pick was available.

Additional samples of fruit were taken for internal ethylene measurement, quality assessment and mineral analysis. Samples were stored in air at 1.5°C for sensory evaluation.

Assessment of fruit quality at harvest

Two ten fruit samples for each orchard and pick date were assessed for quality (six orchards x two samples = 12 samples on each of two picking dates) following storage at ambient for three days. Assessment included:

- Background colour measured at one position on each fruit, avoiding masking pigments, using a Minolta chromameter (L*a*b*);
- Firmness measured by penetrometer at two opposite positions on the equator;
- Total Soluble Solids measured by refractometer of a pooled sample of each ten fruit sample;
- External appearance and internal disorders.

Table 5. Storage treatments

HDC funded storage treatments, included one 15 Kg box for each orchard

	Storage atmos	Temp	Harvest maturity (% starch)	SF?	Box 1	Box 2	Box 3	Box 4	Box 5	Box 6
HDC 1	5%CO_2, 1%O_2	1.5°C	80%	+SF	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F
HDC 2	5%CO_2, 1%O_2	1.5°C	80%	-SF	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F
HDC 3	5%CO_2, 1%O_2	0.5°C	80%	+SF	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F
HDC 4	5%CO_2, 1%O_2	0.5°C	80%	-SF	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F
HDC 5	<1%CO_2, 1.0 %O_2	0.5°C	80%	+SF	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F
HDC 6	<1%CO_2, 1.0 %O_2	0.5°C	60%	+SF	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F
HDC 7	<1%CO_2, 1.0 %O_2	0.5°C	80%	-SF	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F
HDC 8	<1%CO_2, 1.0 %O_2	0.5°C	60%	-SF	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F

Agrofresh funded treatments included one net of approximately 10Kg fruit for each orchard

	Storage atmos	Temp	Harvest maturity (% starch)	SF?	Box 1	Box 2	Box 3	Box 4	Box 5	Box 6
AG 1	3%CO_2, 2%O_2	0.5°C	80%	+SF	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F
			60%	+SF	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F
AG 2	1.5%CO_2, 1.5%O_2	0.5°C	80%	+SF	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F
			60%	+SF	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F
AG 3	<1%CO_2, 1.2 %O_2	0.5°C	80%	+SF in Jan	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F
			60%	+SF in Jan	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F
AG 4	3%CO_2, 2%O_2 increasing to 12% O2 9% CO2 at 1.5°C for 2 weeks prior to opening	0.5°C	80%	+SF	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F
			60%	+SF	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F

Fruit sampling for sensory evaluation

Sensory evaluation was carried out in November 2013 to compare air-stored samples from each orchard and pick date, and in April and June 2014 to assess samples subjected to the full range of storage protocols.

The samples assessed in November had been stored since harvest in air at 1.5°C. They were removed to ambient three days before tasting.

Prior to the sensory evaluation in April half of the fruit stored in treatment AG 4 were removed and placed in another CA cabinet, set to 12% O2 9% CO2 at 1.5°C for conditioning for two weeks. Likewise, prior to the sensory evaluation in June the AG 4 cabinet was reset to the conditioning treatment for two weeks.

Prior to the assessments in April and June the fruit was removed from storage and placed in air at 15°C for three days, and then moved to ambient at 18°C for one or two days before tasting. Tasting was carried out by staff belonging to seven organisations, comprising UK marketing companies, Gala growers or researchers. Thus all tasters had experience of Gala quality over several seasons. Each organisation was provided with randomly labelled fruit from all samples (96 treatment/orchard combinations). In one case in April an organisation received two fruit from each sample (192 fruit). Tastings were carried out over a two day period. No person tasted more than 16 apples at a single session. Thus at least seven assessments were carried out for each sample.

Protocol for sensory evaluation

Tasters were presented with whole, randomly numbered apples and asked to assess each using a 1-10 score using the following criteria.

- Aroma (1 bad - 10 excellent)
- Flavour (1 bad - 10 excellent)
- Sweetness (1 low - 10 high)
- Acidity (1 low - 10 high)
- Firmness (1 low - 10 high)
- Crispness (1 low - 10 high)
- Off Flavours (1 bad - 10 none)
- Overall Acceptability (1 bad - 10 excellent)

In addition tasters were provided with a form to include comments if they wished.

Quality analysis

Simultaneously with the sensory evaluation in April and in June 20 fruit (two 10 fruit samples) from each sample were assessed for quality as described above.

Volatile collection

For selected samples volatiles were collected from whole and quartered apples. In April, this was carried out two weeks after the sensory evaluation on samples that were removed from store two weeks after the sensory samples, but otherwise using the same protocol. In June the volatiles were collected simultaneously with the sensory evaluation.

Five-six apples were weighed and sealed in 3L glass jars for one hour. Volatiles were then collected by pumping air from the jar through a thermal desorption column at a rate of 100 ml/min for five minutes. This process was repeated on the following days, but with the fruit cut into quarters and the jar sealed for only 30 minutes.

Volatile analysis

The volatiles were analysed using a GC mass spectrometer with thermal desorption.

Statistical analysis

Interactions between orchard x picking date for HDC storage regimes and Agrofresh regimes were analysed using Genstat 13.0.

Cluster analysis

Cluster analysis was performed using R programing scripts

Results

Harvest quality and sensory quality of short-term air stored fruit

Table 6 shows the quality analysis at harvest. Fruit from the six orchards and both picking dates that had been air stored for five weeks was subjected to quality analysis (Table 7) and sensory testing (Table 8). Orchard E had the highest overall acceptability for both picks, even though fruit from Orchard D was both firmer and had higher Brix for both picks. The sensory data (Appendix 1) indicates that the assessors considered the fruit from Orchard E had more flavour, better aroma, was “sweeter” and was crisper (except for Pick 2) compared to that of Orchard D.

Table 6. Harvest quality of Gala apples picked from six orchards on two picking dates. Each data point is the mean of measurements on two samples of 10 fruit.

Orchard	Pick 1			Pick 2		
	Firmness (N)	% Brix	CTIFL Starch score	Firmness (N)	% Brix	CTIFL Starch score
A	91.84	10.6	2.8	n.d.	11.4	3.6
B	93.10	10.4	1.6	92.00	11.6	4.6
C	89.30	10.4	1.6	90.76	11.6	4.7
D	103.02	12.0	1.4	98.42	11.8	3.9
E	98.35	10.8	1.4	97.08	12.1	4.0
F	84.67	11.4	3.4	n.d.	11.5	4.1

Table 7. Dry matter (%) content, respiration rate (ml CO_2/kg/h), internal ethylene concentration (ppb) of Gala sourced from 6 orchards over two picking dates

Orchard	Pick 1			Pick 2		
	% Dry Matter	Resp rate	I.E.C. (ppb)	% Dry Matter	Resp rate	I.E.C (ppb)
A	13.7	1.21	129.3	15.2	1.17	777.1
B	14.0	1.16	82.3	14.8	1.48	103.1
C	16.8	1.31	78.6	15.5	1.59	576.8
D	16.5	1.22	372.2	15.9	1.14	824.4
E	14.4	1.32	345.0	14.7	1.53	792.9
F	13.6	1.20	58.6	13.5	1.54	807.4

Orchards C and D had the highest dry matter (DM) content (16.5-16.8% DM) while in orchards A and F the dry matter content was lower (13.6-13.7% DM) in Pick 1 fruit. A similar pattern of DM content was observed in second pick fruit. Fruit with high dry matter at harvest corresponded to higher % Brix at harvest and this remained high during storage.

Internal ethylene concentration (IEC) increased in line with picking date, Gala harvested at Pick 1 from orchards D and E exhibited higher IEC (345-372 ppb) than fruit from other orchards. Moreover, by the second pick the IEC of five out of six orchards averaged (576-834 ppb) with only B remaining low, at around 100 ppb, a concentration considered as the optimum for long-term storage. Fruit respiration remained low (1.16-1.32 ml CO_2 kg h) in Pick 1 fruit rising to 1.14-1.59 (ml CO_2 kg h) by the second pick.

Apples from orchards with a higher dry matter content at harvest led to higher soluble solid content at harvest, which resulted in fruit with a higher % Brix (15.5-16.0%) at harvest that was maintained during the storage season

Fruit with a higher DM content produced fruit that had higher fruit firmness as measured by penetrometer and by sensory evaluation.

The combination of higher fruit firmness and high % Brix made a large contribution towards raising the overall acceptability of fruit tasted.

Table 4 Quality of Gala apples used for November sensory testing. Fruit was picked from six orchards on two picking dates and stored in air at 1.5°C. Each data point is the mean of measurements made on two samples of 10 fruit.

Orchard	Pick	Firmness (N)	% Brix
A	1	69.14	12.65
A	2	76.54	12.60
B	1	62.43	12.55
B	2	69.94	12.65
C	1	68.78	14.15
C	2	68.70	13.80
D	1	72.51	13.65
D	2	74.99	13.10
E	1	66.27	12.70
E	2	69.33	12.65
F	1	66.02	11.70
F	2	69.01	11.75

No rots or disorders were observed.

Table 9. Overall acceptability of Gala apples in November. Samples were tested from six orchards, picked at two harvest maturities, and were air-stored prior to tasting. Each data point is the mean of assessments by 15 tasters

	Pick 1	Pick 2
Orchard A	5.73	5.67
Orchard B	4.87	5.53
Orchard C	5.64	5.93
Orchard D	4.93	6.07
Orchard E	5.87	6.33
Orchard F	5.27	5.00

Quality and sensory acceptability of fruit in April

Samples from all orchards, picks and storage treatments were subjected to quality and sensory assessment in April, after six months of storage. The overall acceptability scores are summarised in Table 10. No statistical comparison of the HDC treatments with the AG treatments has been carried out. However, within each treatment group no treatment differences were observed nor any orchard differences.

Firmness is summarised in Table 11. In this case there were very significant treatment effects. However, at this time there was no clear indication that SmartFresh^{TM} resulted in firmer fruit for Pick 1, but an effect was apparent for Pick 2. There was however a significant orchard effect, with orchards C and D providing firmer fruit. Comparison of storage treatments confirms the earlier observations that low CO_2 does not maintain fruit texture as well as 5% CO2. Firmness of fruit was higher in fruits kept in 5% CO2, 1% O2 (5/1) CA stored at 1.5 (HDC 2) or 0.5°C (HDC 4) compared to fruit stored in 1.0% O_2 <1% CO_2 (HDC 7). The firmness of SmartFresh^{TM}-treated Gala maintained in alternative storage regimes (3% CO_2 and 2% O_2 [3/2]) were similar to 5/1 stored fruit where CO_2 was present in the storage atmosphere. In the scrubbed CO_2 regime firmness was lower.

Figure 1 compares firmness measured by penetrometer with the score of firmness provided by the tasters. There was a significant correlation (R = 0.70) indicating that penetrometer measurement is a useful measure for assessing quality. Figure 2 compares firmness measured by penetrometer with the score for overall acceptability provided by the tasters. The correlation in this case (R=0.37) indicates that firmness is an important criterion for tasters.

% Brix is summarised in Table 12. There were no treatment effects, but a very significant orchard effect, as Orchard C had a consistently high % Brix value, and Orchard F a low % Brix.

Correlations between TSS and overall acceptability were low. Interestingly, the correlation between TSS and sweetness, as determined by tasters, was also low.

The two orchards (C and D where apples were high in % Brix and dry matter content at harvest) scored particularly highly in overall acceptability following storage in 5/1 at 1.5°C and picked at 80% starch with no SF-treatment. Averaged across all six orchards, there was no significant difference in the CA regimes tested on overall acceptability scores.

Table 10. Overall acceptability of Gala apples in April following storage under a range of storage treatments followed by three days at 15°C and two days at ambient. Fruit were picked from six orchards on two picking dates. Each data point is the mean of assessments by seven tasters.

Pick 1

	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F	Mean
AG 1	5.75	5.75	6.00	6.50	6.38	6.63	6.17
AG 2	6.38	5.38	6.50	5.88	5.88	5.63	5.94
AG3	5.71	5.75	6.00	6.38	5.75	5.88	5.91
AG4	6.83	5.86	5.00	6.86	5.43	5.50	5.91
HDC 1	5.57	5.13	6.25	6.25	6.00	5.00	5.70
HDC 2	5.43	5.50	7.25	7.25	4.63	4.86	5.82
HDC 3	6.00	5.63	5.63	5.44	6.00	6.31	5.83
HDC 4	6.38	5.86	6.63	5.13	6.75	5.00	5.96
HDC 5	5.00	4.13	6.14	5.75	6.06	5.25	5.39
HDC 7	4.69	6.13	5.00	6.25	4.98	4.38	5.24
mean	**5.77**	**5.51**	**6.04**	**6.17**	**5.78**	**5.44**	

Pick 2

	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F	Mean
AG 1	6	6.75	5.25	7	6.25	4.71	5.99
AG 2	6	5.43	5.88	5.48	6.13	4.38	5.55
AG3	6.25	5.13	4.43	6.75	5.88	5.68	5.68
AG4	5.07	5.29	6.21	6.43	6.14	6.9	6.01
HDC 6	6.04	6.38	5.75	4.88	6.25	5.75	5.84
HDC 8	5.14	5	3.75	4.75	5.44	5.75	4.97
mean	**5.75**	**5.66**	**5.21**	**5.88**	**6.01**	**5.53**	

Means and results of analysis of variance

HDC Trtmt		Orch		AG Trtmt		Orch		Pick	
HDC1 (P1)	5.72	A	5.53	AG1	6.10	A	6.01	1	6.00
HDC2 (P1)-	5.83	B	5.46	AG2	5.75	B	5.69	2	5.82
HDC3 (P1)	5.82	C	5.81	AG3	5.82	C	5.66		
HDC4 (P1)-	5.97	D	5.74	AG4	5.96	D	6.42		
HDC5 (P1)	5.40	E	5.78			E	5.99		
HDC6 (P2)	5.87	F	5.31			F	5.66		
HDC7 (P1)-	5.25								
HDC8 (P2)-	4.98								
P	N.S.		N.S.		N.S.		N.S.		N.S.
LSD									

Note: - indicates no SmartFresh™

Table 11. Firmness (N) of Gala apples in April following storage under a range of storage treatments, followed by three days at 15°C and two days at ambient. Fruit were picked from six orchards on two picking dates. Each data point is the mean of measurements on two samples of 10 apples

Pick 1

	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F	**mean**
AG 1	90.71	95.68	99.57	97.08	85.39	93.74	**93.70**
AG 2	92.92	93.40	98.85	95.14	89.16	91.67	**93.52**
AG3	83.98	84.23	88.31	90.81	79.21	84.32	**85.14**
AG4	89.38	93.02	97.02	97.59	86.11	88.21	**91.89**
HDC 1	93.49	91.06	97.22	98.47	86.56	92.03	**93.14**
HDC 2	88.70	91.62	96.71	97.99	82.25	89.65	**91.15**
HDC 3	92.26	91.64	99.35	97.66	92.03	92.86	**94.30**
HDC 4	88.96	89.16	92.93	98.57	86.40	88.93	**90.82**
HDC 5	91.87	91.59	97.95	99.17	90.16	94.20	**94.15**
HDC 7	77.15	78.63	80.98	87.53	75.02	83.43	**80.46**
mean	**88.94**	**90.00**	**94.89**	**96.00**	**85.23**	**89.91**	

Pick 2

	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F	**mean**
AG 1	89.61	87.19	90.42	91.80	83.39	88.61	**88.50**
AG 2	90.05	87.75	86.44	88.57	89.16	90.50	**88.74**
AG3	76.22	75.52	71.56	78.17	83.12	80.67	**77.54**
AG4	86.89	85.04	88.67	91.26	87.10	87.78	**87.79**
HDC 6	88.54	88.20	89.32	90.86	89.35	86.00	**88.71**
HDC 8	76.94	72.81	70.12	70.48	67.35	76.23	**72.32**
mean	**84.71**	**82.75**	**82.75**	**85.19**	**83.24**	**84.97**	

Means and results of analysis of variance

HDC Trtmt		Orch		AG Trtmt		Orch		Pick	
HDC1 (P1)	93.15	A	87.24	AG1	91.10	A	87.46	1	91.06
HDC2 (P1)-	91.15	B	86.84	AG2	91.12	B	87.71	2	85.65
HDC3 (P1)	94.32	C	90.56	AG3	81.34	C	90.10		
HDC4 (P1)-	90.83	D	92.61	AG4	89.84	D	91.31		
HDC5 (P1)	94.17	E	83.64			E	85.34		
HDC6 (P2)	88.72	F	87.91			F	88.19		
HDC7 (P1)-	80.43								
HDC8 (P2)-	72.30								
P	<0.001		<0.001		<0.001		0.01		<0.001
LSD	3.27		2.84		2.61		3.19		1.84

Note: - indicates no SmartFresh™

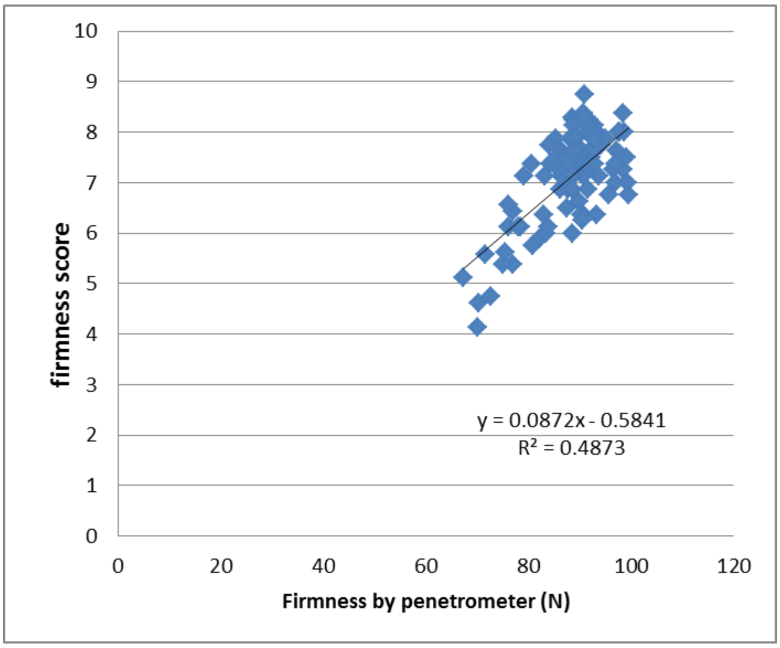

Figure 1. Relationship between firmness score and firmness measured by penetrometer

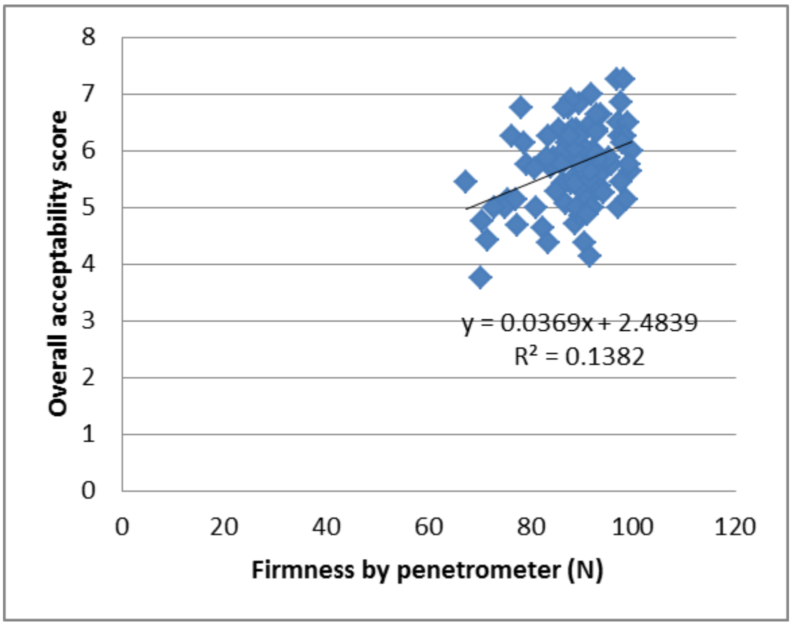

Figure 2. Relationship between overall acceptability score and firmness measured by penetrometer

Table 12. Total Soluble Solids of Gala apples in April following storage under a range of storage treatments, followed by three days at 15°C and two days at ambient. Fruit were picked from six orchards on two picking dates. Each data point is the mean of measurements on two samples of 10 apples

Pick 1

	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F	**mean**
AG 1	12.45	12.15	14.75	13.75	12.50	12.15	**12.96**
AG 2	13.05	12.70	14.60	13.45	13.05	11.95	**13.13**
AG3	13.40	12.35	14.35	13.40	13.05	11.80	**13.06**
AG4	12.80	12.30	14.35	13.60	12.20	11.30	**12.76**
HDC 1	12.50	12.25	14.45	13.55	12.50	12.00	**12.88**
HDC 2	12.35	12.55	14.45	14.00	12.60	12.10	**13.01**
HDC 3	12.60	12.35	14.50	13.35	12.55	12.10	**12.91**
HDC 4	12.85	12.75	14.15	13.80	12.30	12.20	**13.01**
HDC 5	12.30	12.75	14.50	13.65	12.60	12.40	**13.03**
HDC 7	12.95	12.40	13.75	13.30	11.95	11.85	12.70
mean	**12.73**	**12.46**	**14.39**	**13.59**	**12.53**	**11.99**	

Pick 2

Pick	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F	
AG 1	12.95	12.15	14.25	13.10	12.85	12.15	12.91
AG 2	13.05	12.70	13.40	13.40	13.40	11.65	12.93
AG3	14.00	12.90	14.25	13.55	13.15	11.45	13.22
AG4	12.80	13.15	14.15	13.55	12.95	11.30	12.98
HDC 6	12.90	12.50	14.55	13.85	12.85	11.50	13.03
HDC 8	12.40	12.00	13.45	13.35	12.95	12.00	12.69
	13.02	12.57	14.01	13.47	13.03	11.68	

Means and results of analysis of variance

HDC Trtmt		Orch		AG Trtmt		Orch		Pick	
HDC1 (P1)	12.90	A	12.62	AG1	12.98	A	13.09	1	13.00
HDC2 (P1)-	13.03	B	12.48	AG2	13.06	B	12.58	2	13.04
HDC3 (P1)	12.93	C	14.62	AG3	13.17	C	14.30		
HDC4 (P1)-	13.03	D	13.64	AG4	12.89	D	13.50		
HDC5 (P1)	13.05	E	12.56			E	12.92		
HDC6 (P2)	13.05	F	12.02			F	11.75		
HDC7 (P1)-	12.73								
HDC8 (P2)-	12.72								
P	N.S.		<0.001		N.S.		<0.001		N.S.
LSD			0.30				0.38		

Quality and sensory acceptability of fruit in June

The impact of storage strategy was more apparent by June, by which time the fruit had been in store for more than eight months.

The overall acceptability scores are summarised in Table 13. Statistical analysis of HDC and AG treatments were performed separately. However, there was a significant difference between HDC treatments and although there was no significant difference between AG treatments they generally provided higher scores than the HDC treatments. The mean scores for the treatments without SmartFresh™ were all lower than the treatments with SmartFresh™.

Firmness is summarised in Table 14. In this case there were significant treatment orchard and pick effects. By this time there was a clear indication that SmartFresh™ resulted in firmer fruit. All treatments with SmartFresh™ provided firmer fruit than all treatments without SmartFresh™ with the exception of AG 3. Orchards C and D still provided firmer fruit. Orchards C and D, with higher dry matter contents at harvest, also remained the firmest fruit with the highest % Brix during storage.

Firmness appears to be an important criterion for tasters, and the taster assessment relates well to penetrometer values. Figure 3 compares firmness measured by penetrometer with the score of firmness provided by the tasters. There was a significant correlation ($R = 0.77$). Figure 4 compares firmness measured by penetrometer with the score for overall acceptability provided by the tasters. The correlation in this case ($R=0.56$), which is higher than that observed in April, indicates that firmness is an important criterion for tasters.

% Brix is summarised in Table 15. As in April there were no treatment effects, but a very significant orchard effect, as Orchard C had a consistently high % Brix value related to high dry matter content of fruit, and Orchard F a low % Brix, orchard F had the lowest dry matter content (13.7% at harvest).

Correlations between % Brix and overall acceptability were low. However, unlike the April data, there was a significant correlation between % Brix and sweetness as determined by tasters.

The overall acceptability of CA regimes was influenced by orchard. Averaged across all treatments, no significant difference was observed in acceptability scores between Gala kept in 5/1 regimes stored at 0.5 or 1.5°C. Moreover, no effect of SmartFresh™ on overall acceptability score was observed in fruit stored in 5/1.

Gala picked at 80% starch and stored in 5/1 CA (0.5°C) with SF had the highest overall acceptability scores.

When averaged across all orchards the effect of modifying CO_2 or O_2 (AG-treatments) on overall acceptability was similar to storing fruit in 5/1. However, there was a strong orchard interaction with CA regimes and in orchards that had higher dry matter content 3% CO_2, 2% O_2 and 1.5% CO_2, 1.5% O_2 led to fruit with good overall acceptability. Interestingly, storing Pick 1 fruit (80% starch) in 1% O_2 with SF provided fruit of equal acceptability,

No effect of storage temperature was seen in Gala firmness stored in 5/1, Application of SF led to a small increase in firmness retention in Pick 1 fruit (8.7-9.0 kg). However, in later picked fruit (60% starch) SmartFresh^{TM} significantly improved firmness retention (8.8 kg) in fruit stored in 1% O_2, <1% CO_2 compared to untreated fruit (6.1 kg)

Fruit firmness of Gala stored until June was strongly influenced by the dry matter content of fruit with orchards C, D and F retaining better fruit firmness during long-term storage. Picking fruit at 80% starch retained fruit firmness, and storage in 5/1 CA was better for fruit firmness retention compared to storage in 1% O_2.

Table 13. Overall acceptability of Gala apples in June following storage under a range of storage treatments followed by three days at 15°C and two days at ambient. Fruit were picked from six orchards on two picking dates. Each data point is the mean of assessments by seven tasters. An ANOVA was carried out for HDC and AG treatments separately.

Pick 1

	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F	mean
AG 1	5.50	5.25	5.63	6.50	6.00	5.81	5.78
AG 2	6.13	4.61	5.57	5.43	6.29	5.71	5.62
AG3	5.29	6.00	6.43	5.64	6.25	4.88	5.75
AG4	5.57	5.36	6.29	5.00	5.78	4.86	5.47
HDC 1	5.56	5.79	5.71	5.38	6.00	6.25	5.78
HDC 2	5.71	5.38	6.43	4.29	4.88	5.63	5.38
HDC 3	5.83	5.75	5.94	6.22	5.71	6.81	6.04
HDC 4	5.63	4.63	5.50	5.79	5.38	4.63	5.26
HDC 5	6.00	5.63	5.69	5.71	5.06	6.00	5.68
HDC 7	4.17	4.44	4.00	4.29	5.63	4.88	4.56
mean	**5.54**	**5.28**	**5.72**	**5.42**	**5.70**	**5.54**	

Pick 2

	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F	**mean**
AG 1	6.25	5.50	5.00	6.00	6.50	5.63	**5.81**
AG 2	6.25	5.75	5.57	6.00	5.29	5.38	**5.71**
AG3	4.86	4.29	4.36	6.00	5.14	4.63	**4.88**
AG4	5.83	6.06	4.25	6.13	5.00	4.60	**5.31**
HDC 6	5.86	6.50	5.29	6.31	5.71	4.44	**5.69**
HDC 8	3.86	3.33	4.57	4.57	3.75	4.75	**4.14**
mean	**5.48**	**5.24**	**4.84**	**5.83**	**5.23**	**4.90**	

Means and results of analysis of variance

HDC				AG					
Trtmt		Orch		Trtmt		Orch		Pick	
HDC1 (P1)	5.78	A	5.33	AG1	5.80	A	5.71	1	5.66
HDC2 (P1)-	5.39	B	5.18	AG2	5.66	B	5.35	2	5.43
HDC3 (P1)	6.04	C	5.39	AG3	5.31	C	5.39		
HDC4 (P1)-	5.26	D	5.32	AG4	5.39	D	5.84		
HDC5 (P1)	5.68	E	5.26			E	5.78		
HDC6 (P2)	5.68	F	5.42			F	5.19		
HDC7 (P1)-	4.57								
HDC8 (P2)-	4.14								
P	<0.001		N.S.		N.S.		N.S.		N.S.
LSD	0.68								

Note: - indicates no SmartFresh™

Table 14. Firmness (N) of Gala apples in June following storage under a range of storage treatments, followed by three days at 15°C and two days at ambient. Fruit were picked from six orchards on two picking dates. Each data point is the mean of measurements on two samples of 10 apples (divide by 9.6 to convert Newtons to kg).

Pick 1

	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F	**mean**
AG 1	91.21	89.28	93.59	97.30	86.30	89.90	**91.26**
AG 2	88.88	89.82	94.50	96.19	86.56	88.37	**90.72**
AG3	78.66	82.14	85.06	89.79	76.27	79.54	**81.91**
AG4	89.66	90.30	95.81	95.79	83.56	87.65	**90.46**
HDC 1	91.50	92.63	97.57	100.38	88.01	91.01	**93.51**
HDC 2	85.99	88.54	94.11	94.86	84.84	90.41	**89.79**
HDC 3	92.28	92.85	97.15	94.50	85.54	91.60	**92.32**
HDC 4	86.97	87.79	88.34	90.40	85.60	84.91	**87.33**
HDC 5	85.50	90.16	92.02	98.34	92.53	89.74	**91.38**
HDC 7	62.83	70.81	68.66	75.52	63.03	75.33	**69.36**
mean	**85.35**	**87.43**	**90.68**	**93.30**	**83.22**	**86.84**	

Pick 2

	Orch A	Orch B	Orch C	Orch D	Orch E	Orch F	**mean**
AG 1	85.28	84.61	80.04	83.76	86.94	83.58	**84.03**
AG 2	80.36	79.20	80.25	89.64	84.25	84.89	**83.10**
AG3	73.37	75.49	67.45	65.53	73.17	81.39	**72.73**
AG4	86.16	80.44	81.01	86.73	84.38	86.69	**84.23**
HDC 6	87.33	84.80	87.64	92.26	90.71	85.98	**88.12**
HDC 8	61.48	58.52	58.84	56.54	59.53	71.18	**61.01**
mean	**78.99**	**77.18**	**75.87**	**79.08**	**79.83**	**82.28**	

Means and results of analysis of variance

HDC Trtmt		Orch		AG Trtmt		Orch		Pick	
HDC1 (P1)	93.51	A	81.73	AG1	87.65	A	84.20	1	88.59
HDC2 (P1)-	89.79	B	83.26	AG2	86.91	B	83.91	2	81.02
HDC3 (P1)	92.32	C	85.54	AG3	77.32	C	84.71		
HDC4 (P1)-	87.33	D	87.85	AG4	87.34	D	88.09		
HDC5 (P1)	91.38	E	81.22			E	82.68		
HDC6 (P2)	88.12	F	85.02			F	85.25		
HDC7 (P1)-	69.36								
HDC8 (P2)-	61.01								
P	<0.001		0.007		<0.001		ns		<0.001
LSD	4.24		3.67		3.10				2.25

Note: - indicates no SmartFresh™

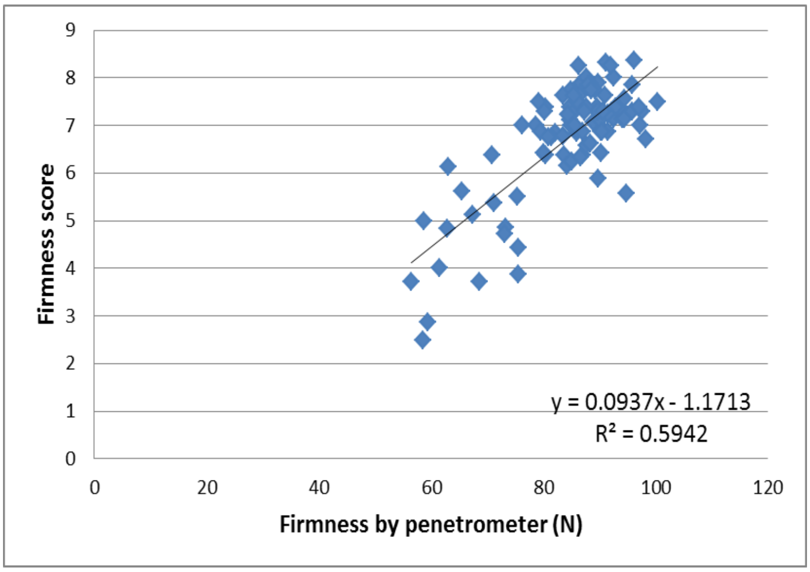

Figure 3 Relationship between firmness score and firmness measured by penetrometer.

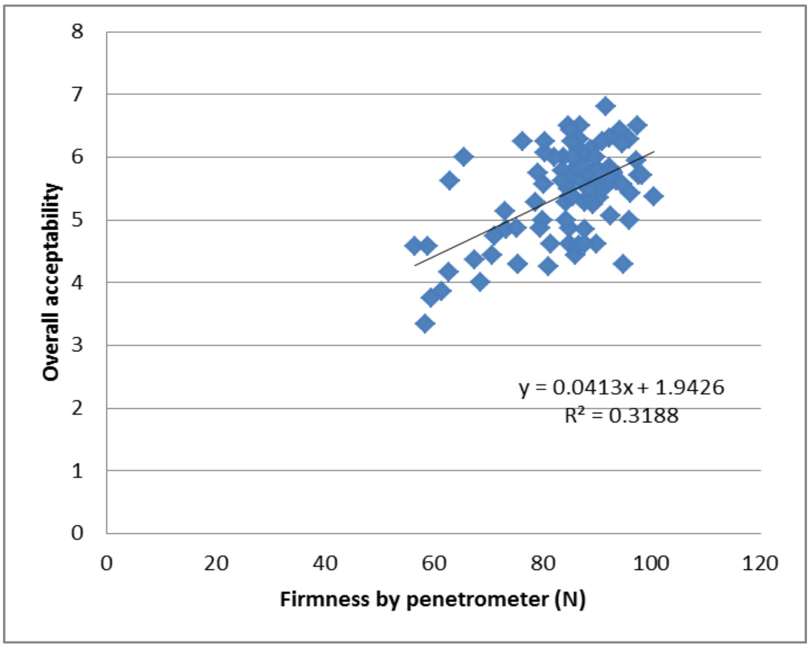

Figure 4 Relationship between Overall acceptability score and firmness measured by penetrometer.

Table 15. % Brix of Gala apples in June following storage under a range of storage treatments, followed by three days at 15°C and two days at ambient. Fruit were picked from six orchards on two picking dates. Each data point is the mean of measurements on two samples of 10 apples

Pick 1

Pick	Barnyard Orch A	Ivyhouse Orch B	Moonfield Orch C	PF2 Orch D	Pumphouse Orch E	Sycamore Orch F	**mean**
AG 1	12.90	12.85	15.00	13.60	12.40	12.05	**13.13**
AG 2	12.65	12.60	14.45	13.65	12.40	11.85	**12.93**
AG3	12.35	12.20	14.55	14.25	12.50	12.10	**12.99**
AG4	12.25	12.15	14.85	13.40	12.20	11.65	**12.75**
HDC 1	12.20	12.25	14.20	12.90	12.15	11.90	**12.60**
HDC 2	12.30	12.40	13.95	13.50	12.35	11.80	**12.72**
HDC 3	12.10	12.35	14.10	13.20	12.40	11.55	**12.62**
HDC 4	11.95	12.00	13.75	12.95	12.70	11.65	**12.50**
HDC 5	12.60	12.25	14.30	13.55	12.65	11.75	**12.85**
HDC 7	12.25	12.10	13.95	13.50	11.95	11.55	**12.55**
mean	**12.36**	**12.32**	**14.31**	**13.45**	**12.37**	**11.79**	

Pick 2

	Barnyard Orch A	Ivyhouse Orch B	Moonfield Orch C	PF2 Orch D	Pumphouse Orch E	Sycamore Orch F	**mean**
AG 1	12.95	12.70	13.85	13.45	13.40	11.15	**12.92**
AG 2	12.45	12.30	13.80	13.55	12.95	11.60	**12.78**
AG3	12.40	12.25	13.30	13.10	13.35	11.65	**12.68**
AG4	13.00	12.15	13.95	13.35	12.60	11.25	**12.72**
HDC 6	12.50	12.20	14.05	13.90	13.15	11.35	**12.86**
HDC 8	13.40	12.10	14.05	13.05	12.75	11.80	**12.86**
mean	**12.78**	**12.28**	**13.83**	**13.40**	**13.03**	**11.47**	

Means and results of analysis of variance

HDC Trtmt		Orch		AG Trtmt		Orch		Pick	
HDC1 (P1)	12.60	A	12.41	AG1	13.02	A	12.69	1	12.95
HDC2 (P1)-	12.72	B	12.21	AG2	12.85	B	12.40	2	12.77
HDC3 (P1)	12.62	C	14.04	AG3	12.83	C	14.22		
HDC4 (P1)-	12.50	D	13.32	AG4	12.73	D	13.54		
HDC5 (P1)	12.85	E	12.51			E	12.72		
HDC6 (P2)	12.86	F	11.67			F	11.66		
HDC7 (P1)-	12.55								
HDC8 (P2)-	12.86								
P	n.s		<0.001		n.s.		<0.001		n.s.
LSD			0.29				0.32		

Note: - indicates no SmartFresh™

Developing a protocol for assessing Gala sensory acceptability in terms of volatiles and flesh texture/composition

One of the objectives of this project is to define a practical protocol for assessing Gala sensory quality in terms of measurable characteristics such as volatiles and flesh texture/composition. As part of this process an analysis of volatiles produced by whole and cut fruit was undertaken to relate volatile profiles to the sensory analysis. Volatiles were collected from whole fruit of 48 of the 96 samples and from cut fruit of 28 samples at the same time as the sensory analysis in each of April and June.

The range of volatiles detected depends to some extent on the method of volatile capture. The main volatiles detected consistently in this study (both assessment times, whole and cut fruit) were:

- Ethylhexanol
- Hexadecane
- Methylbutylacetate
- Benzaldehyde
- Benzyl alcohol
- Hexanol
- Decanal
- Butylbutyrate
- Benzoic acid
- Hexyl 2-methylbutyrate

Importantly, hexyl acetate a volatile related to apple flavour was only detected in April samples but not in later samples. Interestingly hexanol, the precursor to hexyl acetate, was detected in Gala late in the season which may suggest that the later stages of volatile synthesis (esterification) have been degraded with length of storage.

It will be necessary to validate and optimise the volatile collection system through the whole Gala storage season in 2014/15.

The 2014 volatile data, together with firmness measured using a penetrometer and % Brix measured using a refractometer, were used to carry out a "cluster analysis" of the apple samples. This analysis used combined data from assessments carried out in April and June. The analysis indicated that the samples fall into five clusters as illustrated in Figure 5.

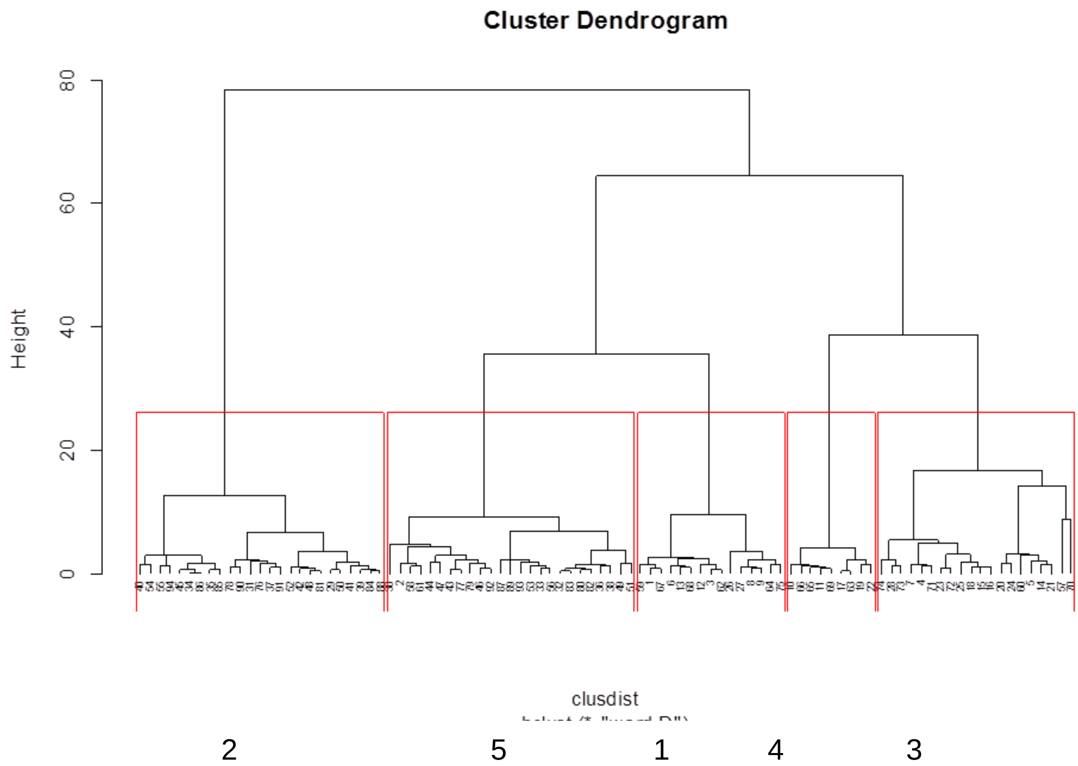

Figure 5 Cluster analysis undertaken for Gala samples assessed for quality in April and June on the basis of volatile production of whole apples, firmness and TSS

Although the identification of the clusters did not use the sensory data, the samples from the different clusters differ significantly in their *Overall Acceptability* scores; those in Cluster 4 have the highest *Overall Acceptability*, while those of Cluster 5 have the lowest. This ranking of *Overall Acceptability* between clusters is the same as the ranking of *Flavour* score. Table 16 and Figure 6 show the average characteristics of each cluster. It is notable that Cluster 4 has high hexanol and decanal production and low ethyl hexanol and hexdecane production/accumulation compared to Cluster 5. Cluster 4 also has the highest % Brix value.

This analysis indicates the potential for using measureable characteristics to assess sensory qualities. This approach will be developed further in Phase 2.

Table 16. Volatiles concentrations, Firmness and TSS and Consumer scores for Overall Acceptability and Flavour for each of five clusters identified among Gala samples

cluster	Ethyl hexanol	Hexa decane	Methyl Butyl acetate	Benz aldehyde	Benzyl alcohol	Hexanol	Decanal	Butyl butyrate	Benzoic acid	Hexyl 2-methyl butyrate
					ng/Kg					
1	6.88	73.61	95.19	2.45	23.57	1.50	3.19	1.69	4.86	3.53
2	8.78	77.32	17.16	2.17	0.34	1.29	5.37	0.41	3.71	1.27
3	20.62	27.19	12.35	51.70	13.65	10.96	11.14	16.46	5.31	8.01
4	1.03	1.55	8.97	0.24	0.11	26.20	1.02	0.40	0.10	5.24
5	269.66	80.31	23.53	2.02	0.18	0.69	4.13	0.20	2.10	0.63
Mean	**79.64**	**59.60**	**29.50**	**12.53**	**6.82**	**5.61**	**5.50**	**3.97**	**3.46**	**3.28**

cluster	Firmness	TSS	Overall acceptability	Flavour
1	87.11	12.83	5.68	5.33
2	85.86	12.86	5.66	5.26
3	86.64	12.57	5.56	5.05
4	85.82	13.17	5.88	5.52
5	84.88	12.59	5.25	4.86
Mean	**85.96**	**12.75**	**5.55**	**5.14**

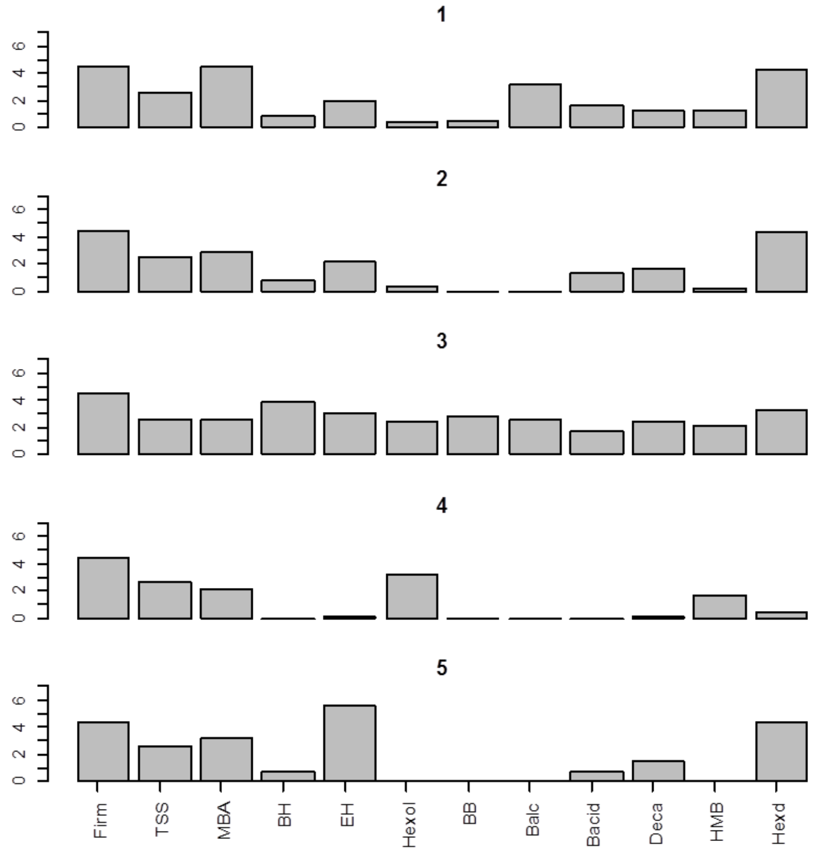

Figure 6 Relative values for twelve characteristics of five clusters of Gala samples. Firm = firmness measured by penetrometer, TSS = total soluble solids measured by refractometer, MBA = Methylbutylacetate, BH = Benzaldehyde , EH = Ethylhexanol, Hexol = Hexanol, BB = Butylbutyrate, Balc = Benzyl alcohol, Bacid = Benzoic acid, Deca = Decanal, HMB = Hexyl 2-methylbutyrate, Hexd = Hexadecane.

Discussion

The first year of this trial has confirmed the importance of dry matter content in maintaining the overall quality of fruit, with a strong relationship between high dry matter content and high % Brix and firmness in assessments of fruit quality performed in April. As storage was extended to June the relationships were still observed, but not considered to be of such importance. Early work by Perring in 1984 reported that Cox with higher dry matter content increased fruit firmness and more recently by Palmer et al (2010) in an extensive study of Gala and Scifresh orchards that a strong correlation of dry matter content and % Brix (soluble solids) was observed in fruits measured after 12 weeks storage. Interestingly, in this trial dry matter content was more closely related to % Brix in assessments made in November (six weeks) and April (5.5 months) than the % Brix measured at harvest. This suggests that the strength of the correlation is dependent on all the starch being converted into sugars which occurs during the first three-four weeks of storage., Fruit firmness and % Brix had a strong influence on the overall acceptability scores in fruit assessed in April but less so in later stored fruit.

The amount of dry matter content of fruit is related to the accumulative amount of light interception of a tree over growing season. Tree architecture plays a significant role in manipulating light capture and thus dry matter accumulation. Crop load also has an important bearing on dry matter, with trees under thinning management having improved dry matter content, improved fruit firmness and higher % Brix (McArtney 1996).

The influence of storage regime on overall acceptability was less pronounced. Storage in 5% CO_2, 1% O_2 extended the storage life of Gala compared to fruit stored in 1% O_2 <1% CO_2 and confirms previous work which identified the termination date of February for Gala stored in 1% O_2 and late March for Gala stored in 5% CO_2, 1% O_2.. Incorporation of elevated CO_2 in the storage atmosphere improves storage retention. Even when modest amounts of CO_2 were added as part of the Agrofresh trial (1.5% and 3% CO_2) an improvement in firmness was observed. The application of SmartFresh^{TM} was most effective when used with fruit stored in 1% oxygen that soften more quickly.

In previous trials (Stow and Genge 2000) it was reported that while Gala stored in 5/1 CA regime improved firmness retention, especially after shelf-life, however, the elevated CO_2 in the storage environment prevented ester formation, notably hexyl acetate and 2-methyl-butyrate. In these trials the presence of hexanol, a precursor to hexyl acetate formation, appeared to be higher in the cluster of apples that appeared to have higher overall acceptability and virtually absent from fruit that scored the lowest. Hexyl acetate is considered to provide a distinctive apple aroma and is commonly reported to be present in large amounts in Gala apples (Plotto et al 1999, 2000 and Stow and Genge 2000).

Conclusions

- There is a strong influence of orchard factors on the eating quality of long-term stored Gala.
- High dry matter content in Gala led to fruit with increased % Brix content and better firmness retention during storage.

- Fruit with higher % Brix and firmness were generally considered to have better overall acceptability.
- Untreated Gala at 85-80% starch background provided fruit with a better eating quality than later picked fruit in long-term storage.
- Later picked fruit (60 % starch) from some orchards treated with SmartFresh™ were able to retain acceptable firmness and eating quality in April and June
- Storing Gala in 5% CO_2, 1% O_2 either at 0.5 or 1.5°C retained similar eating quality in April and June.
- Gala stored in 1% Oxygen with SmartFresh™ were in general of similar eating quality to 5/1 stored fruit.
- Alternative storage regime of 3% CO_2 and 1% O_2 and 1.5% CO_2 and 1.5% O_2 treated with SmartFresh™ provided fruit of good eating quality in April and June.

Knowledge and Technology Transfer

The project was presented at the Marden Fruit Show/EMRA Top Fruit Day in March 2014

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