

An Investigation Into The Fruit Firmness Properties of Some Progeny and Cultivars of Red Raspberry (*Rubus Idaeus*)

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Abstract: Raspberries (*Rubus idaeus* L.) are popular and a major UK soft fruit crop. Several efforts have been geared towards developing an improved cultivar that will have a longer shelf-life. A 1 to 2 days improvement in fruit shelf-life would increase the value of harvested fruit and reduce waste. This research work investigated the firmness characteristics of 22 progeny (clones) derived from a Glen Moy x Latham mapping population by recording the various hardness values at 4°C over a 7 day storage period. A QTS-Texture Analyser was used to measure hardness values and the results were analyzed using Restricted Maximum Likelihood (REML) analysis. The parameters measured were: hardness, hardness1_workdone, final load, and sample length (diameter). Overall, there were significant differences ($p < 0.001$) in the total hardness measurements between progeny. This research shows that some red raspberry clones from Glen Moy x Latham mapping population can stay wholesome after 7 days of storage at 4°C. This is a good development for red raspberry processors and the supermarkets.

Keywords: shelf life, progeny, cultivar, red raspberry, hardness

I. Introduction

Raspberries grow wild in many cold and temperate regions, often at the edges of forests or within glades. Good berry production requires a relatively cool summer and moderate winter temperatures. Preferably humid but well-drained soils are particularly suitable for raspberry cultivation. Intensive areas of commercial production include North Western U.S. states and British Columbia, Eastern Scotland and Southern England. Production on a limited scale occurs in Eastern Canada and the North Eastern United States, as well as New Zealand, Australia, Chile, the USSR and Eastern Europe [1]. Berry production requires a winter dormant period and the fruit may be red (most varieties), blue or black in color; flavor is acidic and aromatic in character [2]. In current crop breeding, the main characters considered in selecting commercial varieties are suitability for mechanical harvesting, shelf life, yield, and resistance to pests and disease. Less attention had been given to the flavors in the past although this is now of greater importance in both fresh fruit and raspberry products [2].

The fragile nature of red raspberries (*Rubus idaeus*) is a key factor that makes it difficult for growers and processors to handle [3]. The ease at which the fruit ruptures during picking, transportation, and on the shelf of supermarkets and other commercial operations are extreme [2,4]. The fact that red raspberries continue to respire and soften after harvest produces a very short shelf-life of 1-3 days [5,6,7]. Like other berry fruits, softening remains the main cause of post harvest waste and loss of revenue due to limited storage, transport and marketability [3,8,9]. In addition, soft raspberries are highly susceptible to gray mould (or *Botrytis*) fruit rot which is the most common and serious disease of the *Rubus* species worldwide.

Recent gene manipulations in red raspberries [10] have increased the potential of developing a cultivar with an extended shelf-life and firmer structure. However, very little research has been carried out on the changes accompanying the ripening process, rate of ripening and resistance to impact (i.e. firmness) of different progenies of red raspberries. Fruit softening is a complex process and is largely dependent on the co-ordinated, interdependent activities of many genes and their regulation and action under differing environmental conditions. Progressive loss of firmness during fruit ripening in raspberry is associated with a loss of skin strength, the separation of the drupelets from the receptacle and a breakdown of cell walls in the mesocarp. During fruit expansion, the thin walled mesocarp cells-hypodermis and epidermis become distended and the delicate nature of the cell walls of the red raspberry contributes to a change in texture [2]. During softening there is a rapid collapse of the cell wall and the total pectin content is halved during maturation [11]. Many cell wall changes are common to most ripening fruits and modifications to the polysaccharide components of cell walls. For example, cell wall modifying enzymes, such as β -galactosidase and expansins are thought to act early in the ripening process but may restrict or control the activities of other ripening-related enzymes such as polygalacturonases (PG), pectinmethylesterases (PME) [12,13]. Increased activities of ripening enzymes (hydrolases) in the cell wall such as polygalacturonase and endo-1, 4- β -D glucanase (cellulase) usually accompany cell wall degradation [12]. In an effort towards obtaining more information about the ripening

process of red raspberry, *Sexton et al.*, (1997) measured the skin strength loss accompanying ripening in *Rubus idaeus* L. cv Glen Clova and this was linked to the morphological changes in the fruit.

Raspberry plants (*Rubus idaeus* L; family Rosaceae) are 1–2m in height and generally have twigs with many short thorns, compound foliage and pinnate leaflets [2]. Leaves are white underneath and light green above. Red raspberries have biennial tops with first-year shoots (primocanes) developing either from vegetative buds on the perennial root system or basal buds of the second-year canes (floricanes). The florescence is a simple panicle with white flower of short and linear petals. Aggregate fruits are composed of plugs (receptacle) and many drupelets [2].

So, the primary aim of this work was to examine the hardness (or firmness) characteristics of a range of progeny derived from a “*Glen Moy*” x “*Latham*” mapping population by recording specific ‘Hardness’ measurements during storage (4°C) over 7 days. This will aid recommendations of fruit firmness scores required to maintain fruit quality during transit and storage through the supply chain to be made.

II. Materials And Methods

2.2 Fruit Samples

Genetics research has focused on the development of mapping populations to generate markers and create genetic linkage maps that form the basis for linking phenotype (plant traits) to genotype (plant genes) in important crop species. A full-sib family derived from the cross between the North American red raspberry cv. ‘Latham’ and European red raspberry cv. ‘Glen Moy’ was designed in the year 2010 at The James Hutton Institute, Dundee [formerly known as Scottish Crop Research Institute (SCRI)., Dundee] and used for the purpose of this research. The progeny developed from the crossing of *Latham* x *Glen Moy* differs widely in a number of important characteristics such as: colour, size and time of ripening (i.e. when fruits can be picked across all progeny).

The progeny from this cross also segregates for fruit firmness/softness and shelf-life and so this population provides an excellent opportunity to investigate different shelf-life characteristics. ‘Latham’ is small in size, softer and has a deep red glossy color. It sets fruit late in the season and it is also characterized with a sweet aromatic taste. In contrast, ‘Glen Moy’ has large fruits, which are firmer but pale in colour. ‘Glen Moy’ also has a sweet flavour character. Ripe fruit from *Glen Moy* has been assessed as being twice as firm as Latham [2].

2.2 Mapping Population

The mapping population consists of 188 progeny derived from crossing ‘Glen Moy’ and ‘Latham’. A randomized complete block trial was used in planting the population with each sample in triplicate. The raspberries were grown under polytunnels at the James Hutton Institute, Invergowrie, Dundee, United Kingdom. The planting site was covered by 150-µm thick visqueen polythene and consisted of three rows/bay in the tunnel, 2.5 meter gap between rows and a 2-8m leg row. Weeds were controlled by covering the leg rows with UV-stable fabric mulch (phormasol). Fertilization and irrigation were controlled by a D8 Dosatron water powered dosing system fed through Ram light tape under the bedding polythene.

2.3 Preliminary Hardness Measurements

Preliminary trials were carried out with Raspberries purchased from a supermarket in order to ascertain an appropriate length of storage time in the cold store (4°C) for monitoring firmness and shelf-life characteristics. The potential for doing experiments with a shaker in order to mimic transport of fruit from the grower to supermarket was also investigated with the same samples. Two punnets each of cultivars *Glen Ample* and *Tulameen* grown from the same region and with the same “use by” dates. A total of ~30 raspberries from each cultivar were transferred into new 40 ml labeled plastic punnets to create a single layer of fruit for the purpose of each experiment. The ‘hardness’ of fruit (6 individuals) from each punnet was measured using the QTS-25 Texture Analyzer (Brookfield Engineering, UK) on the day of purchase (Time = 0), and then after storage in a cold room at 4°C initially over a period of 1 to 6 days. A separate experiment involved shaking three replicate punnets of each cultivar at 400 rpm at room temperature and at 4°C, and removing a punnet over a period of 0h (Control), 2, 4, and 24h to collect 10 individual fruit for determining the hardness values. The same trials were also carried out with two clones (R81 and R173) from the *Latham* x *Glen Moy* mapping population when fruit had become ripe; the shaking experiment (R81) involved taking measurements over 0, 1, 2, 3, and 7 days, and the storage experiment (R173) was monitored after 0, 4, 24, and 28h treatment.

For the purpose of the main study, 22 samples of different ripe progeny with a range of ‘Firmness’ scores (high, medium, soft) plus both parents (Latham and Glen Moy) were selected based on previous measurements where softness was assessed by a subjective score of firmness (‘Breeders score’) on a 1-4 scale (1=firm; 4= very soft), which has been validated by statistical analysis as being appropriate for mapping this trait. The Breeders score was also correlated with the QTS-25 machine measurements of hardness,

hardness1_workdone, and final load during the same season, so these parameters were also measured. Fruit was manually picked from three replicates over a period of two weeks and all replicate samples were collected on the same day and put into labeled plastic punnets and temporarily stored in cold boxes until transport to the cold room (4°C). Generally, enough fruit per plant (20-40; depending on size) were collected to form a single layer in a punnet. After collection, the fruit was transported by van in the cold box for a period of 25 minutes via a set route (4 miles; 6.4 km) to simulate the transport process from a commercial grower to supermarket, and hence, introduce impact damage effects on the raspberries.

2.4 Quantitative Measure of Firmness Using a QTS-25 Texture Analyser

The hardness of individual raspberry fruit was measured using a compression method with a QTS-25 Texture Analyser (Brookfield Engineering, UK). The work done per unit weight of berry at a set compression level was determined using a compression plate which mimics the pressure applied to berries in the punnet. The texture analyser was fitted with a 25 Kg load cell to drive an aluminum probe of diameter 38 mm and length 80 mm. The trigger point was set at 0.049 Newtons (5 Kg) and the probe used at a speed of 30 mm/min. The individual fruit was positioned on the plate lying sideways and the parameters measured by the QTS-25 were Hardness (Newtons, N), Hardness1_workdone (N/sec), Final load (N) and Sample length (Diameter, mm). During operation of the QTS-25, the probe was driven down perpendicular to the surface of the fruit drupelets. On making contact with the fruit, a gradual increase in work done was recorded on force traced x distance travelled by the probe. This was then followed by a second gradual drop of the probe to finally compress the berry at a 30% deformation. The peak force applied to compress the berry is the skin strength at that percentage deformation. The compression in this test was defined as the force required to close the opening of the raspberry positioned on its side by applying a perpendicular force to the drupelet long axis.

Ten berry weights (g) from ripe fruit collected from each progeny were measured on the day of harvest. Six individual fruit were tested from each progeny in triplicate. Measurements were taken on the day of harvest (0 day), 2, 4 and 7 days after harvest. After each day of measurement, the remaining samples were returned to the cold room (4°C).

2.5 Statistical Analysis

The results were analysed using (REML) Restricted Maximum Likelihood (Mixed Modeling). The REML deals with more than one error term. It is capable of removing nuisance parameters and outliers. The significant difference was measured by comparing results within each group. Progeny are considered significantly different as the mean difference of hardness values > x2 Standard Error Difference (SED)

III. Results And Discussion

3.1 Preliminary Experiments for Storage and Transport of Raspberry

In this experiment, we used the QTS-25 Texture Analyzer to measure the fruits hardness, hardness workdone and final load. The mean hardness value of *Glen Ample* was 101N and 88N on day 0 and on the sixth day respectively (Fig. 1), while *Tulameen* had a mean hardness value of 85N and 62N on day 0 and day 6, respectively (Fig. 2). We observed the same pattern from the shaking experiment at 4°C, where a gradual decline in hardness measurements was detected over the period of shaking from 2 to 28hours. For *Glen Ample*,

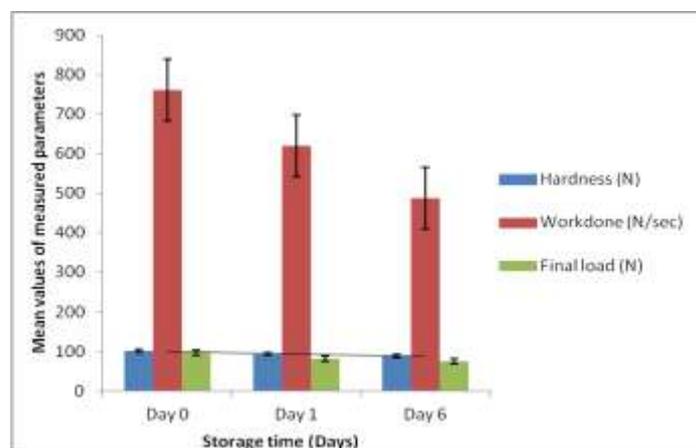


Fig.1. Storage at 4°C over time of cv. *Glen Ample*

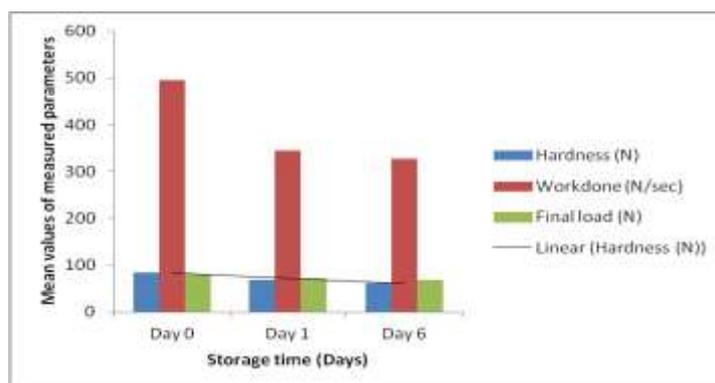


Fig.2. Storage at 4°C over time of cv. *Tulameen*

The mean hardness was 87N after 28hours from the initial 113N (Fig. 3) and *Tulameen* recorded a mean hardness of 55N after 28hours from the initial 83N (Fig. 4). From both storage and shaking experiments, we observed that aside the fact that *Glen Ample* was the firmer cultivar before storage (at day 0), it was also able to retain its firmness (hardness) better than *Tulameen* over time. A reduction in firmness of (13N: storage, 26N: shaking) and (23N: storage, 28N: shaking) was measured for *Glen Ample* and *Tulameen* respectively during both experiments. So, we concluded that *Glen Ample* was a slightly firmer variety than *Tulameen* when measured over 1 and 6 days storage at 4°C, and this also also indicates a longer shelf-life property for *Glen Ample*.

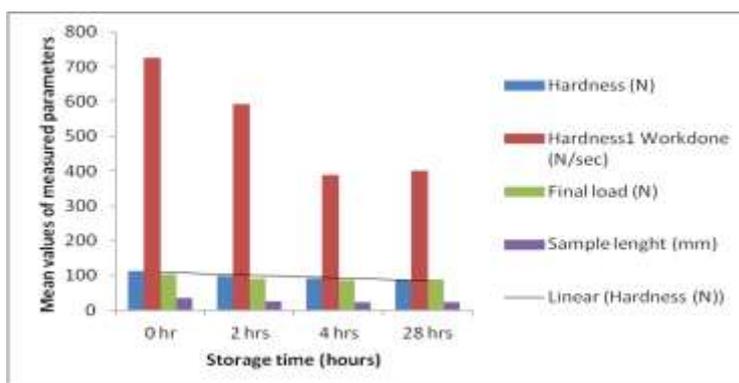


Fig.3. Shaking time while being stored at 4°C over time with *Glen Ample*

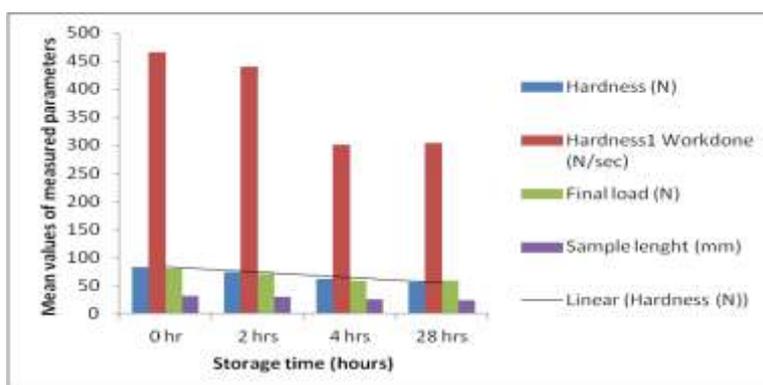


Fig.4. Shaking time while being stored at 4°C over time with *Tulameen*

The result above is similar to the works of [Banados et al. \(2002\)](#) where they noticed a reduction in firmness of fourteen (14) cultivars of red raspberry which includes *Tulameen*, the samples were kept under refrigeration condition over few days.

This was in contrast to the experiment carried out at room temperature as hardness values were greater over the periods of shaking from 2 to 22h compared to the control values (T=0, no shaking) for both *Glen Ample* and *Tulameen*. Although, this was not expected, the examination of the fruit at the end of the shaking

experiment indicated that they were slightly drier in appearance indicating loss of moisture. This loss of moisture would therefore contribute to an increased hardness.

The same shaking (transported) experiment at 4°C using progeny R81 from the mapping population also followed the same pattern as the supermarket fruit shows a gradual decrease in hardness measurements over the period from 4 to 28h incubation with mean hardness dropping from initial 65N (0hr) to 42N after 28h (Fig 5). This however constitutes about 35% loss of firmness over the 28h period. The storage experiment with clone R173 also showed a steady decline of hardness measurements of 97N, 95N, 61N and 60N over the sampling period of 1, 2, 3 and 7 days respectively from the Day 0 firmness value 99N at 4°C, with a more significant decrease indicated after Day 2 storage (Fig. 6). Frederic *et al.* (1992) reported that the loss in fresh mass during storage may be due to the respiratory activity which in turn is enhanced by ethylene production.

Overall, these preliminary storage and shaking (transport) experiments showed a similar pattern of a decrease in hardness over time and indicated that the QTS-25 Texture Analyzer was suitable for measuring the differences in firmness of raspberry fruit during a time course study and also provided information about the most useful periods of incubation to monitor. The QTS-25 Texture Analyser measurements for hardness, hardness1_workdone, and final load followed the same pattern during the period of analysis. Storage experiments at 4°C were subsequently carried out with progeny from the mapping population over a period of 7 days.

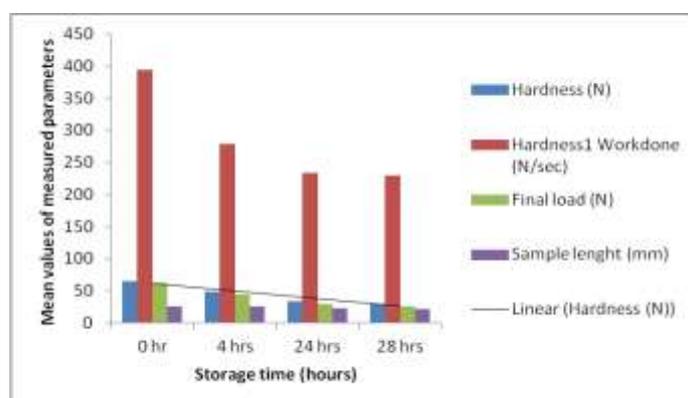


Fig.5. Transport at 4°C over time with progeny R81

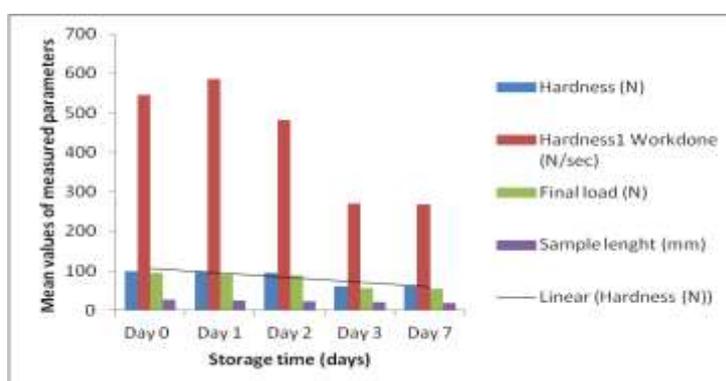


Fig.6. Storage at 4°C over time with progeny R173

3.2 Shelf-Life Characteristics Of A Range Of Progeny Derived From A Glen Moy X Latham Mapping Population

Restricted Maximum Likelihood (REML) was used in the analyses of the data collected after storage to deal with more than one random error term, and sample_length (diameter of fruit) was included as a covariate. The specific sources of variability and fixed effects selected were Cultivar (Cv), Day (period of storage at 4°C), and the Day_Cultivar interaction. Initial analysis demonstrated that all the parameters of hardness, hardness1_work done, and final load measured were highly correlated (Fig. 7), so subsequent analysis was only performed across all samples for hardness. Overall, there were significant differences ($p < 0.001$) in the total hardness measurements between progeny when sample_length, day, and cultivar were considered (Table 1). This is similar to the observation of Hall and Stephens, 1999 during the assessment of fruit firmness of some Red Raspberry cultivars using destructive methods such as sonic testing and slump testing, they observed that there was a high degree of variability in results obtained from the firmness tests.

REML analysis indicated that there was significant differences between many of the samples when the overall mean hardness values were compared (Table 2; significant when mean value >x2 SED). When Moy (hard) was compared to Latham (soft) and all progeny, there was a significant difference in all cases except for R42, R73 and R252 (Table 2). No significant difference existed among all the cultivars (clones) except when compared to R42, R73 and R252 (Table 2). Thus, these three clones (R42, 73 and R252) showed a similarity to the parent Moy in terms of hardness and shelf-life properties. Clone R42 showed the highest mean value of hardness and therefore would be of interest for future studies into the genetic and physiological basis for this increased firmness. Mean hardness values for most clones were not significantly different from the parent Latham, except for R42, R73, R252, as well as additional clones R104, R171 and R184. Thus, among the 22 mapping clones investigated, six clones (R42, R73, R252, R104, R171 and R184) potentially showed longer shelf-life properties.

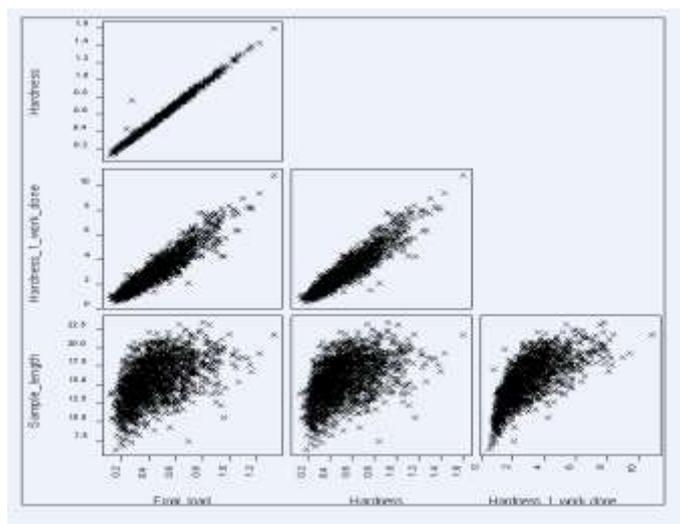


Fig.7. Scatter diagram of correlations between QTS-25 Texture Analyser measurements for hardness, hardness1_workdone and final load.

Table 1. REML analysis using fixed effects of cultivar, day and the interaction between these for the total hardness measurements

Fixed term	Wald statistic	n.d.f	F-statistic	d.d.f	F pr
Sample length	453.51	1	453.51	782.8	<0.001
Day	80.75	3	26.92	146.0	<0.001
Cultivar	224.82	23	9.77	45.3	<0.001
Day.Cultivar	108.52	69	1.57	139.2	<0.013

Note: Sample_length (diameter of fruit) included as a covariate.

F pr column gives the p values for the fixed effect terms: Day, Cultivar & the interaction between Day and Cultivar.

Table 2. Predicted means of hardness measurement

Cultivar	Mean of Hardness measurement for all Cultivars
Latham	0.4266
MOY	0.7251
R1	0.4941
R13	0.4179
R42	0.7361
R56	0.4134
R61	0.4524
R62	0.4885
R66	0.4905
R73	0.7081
R88	0.4831
R93	0.5090
R102	0.4173
R104	0.5354

R114	0.3843
R136	0.3897
R171	0.6101
R184	0.5893
R214	0.4553
R234	0.4726
R241	0.5715
R248	0.3940
R252	0.7078
R254	0.4370

Note: Standard errors of differences (SED)

Average: **0.05086** ($>x2= 0.10172$)
 Maximum: 0.05738
 Minimum: 0.05008
 Average variance of differences: 0.002589

When predicted means of hardness for the interaction between Day_Cultivar were compared, the parent Moy and clones R42, R73, R241, and R252 were significantly firmer than all other clones on the first day of measurement (Day 0), but after 7 days of storage the hardness values had significantly reduced and no significant differences were detected among all progeny (Table 3). This suggests that despite an initial high firmness prior to storage of clones R42, R73, R241, and R252, there appears to be an efficient and high activity of genes involved in the softening process at 4°C during storage for up to 7 days in these samples. Although, Raspberry are classified as non climacteric fruits based only on skin colour change after harvest [18], but, it is important to also to note that red raspberry can also be classified as climacteric fruits based on continuous production of ethylene after harvest which consequently results in the internal ripening of the fruit and gradual degradation of the cell wall pectin [2]. Further research could focus on steps to reduce the activities of the genes involved in softening in order to maintain a desirable hardness (i.e. firmness) over a 7 day period of storage. For all the other clones which showed reduced hardness values at the start (Day 0) and maintained them at the end of the 7 day storage period, further research could be carried out to compare the activities of implicated softening genes with those in the firmer clones. Fig. 8 shows the decline of mean hardness values for all cultivars (clones) during storage over time at 4°C. In summary, of the 22 clones investigated, only three clones R73, R241, and R252 plus Moy which are classed as ‘firm’ showed a significant reduction in firmness after 7 days of storage at 4°C, indicating that further research on the remaining 19 clones may lead to the identification of factors and genes important in ensuring a supermarket shelf life of up to 8 days and maintaining wholesomeness.

Fruit softening remains the main cause of post harvest waste and lost revenue in all soft fruit with financial losses at the farm gate into six figures in a poor season for strawberry and raspberry [2,1]. This currently costs the industry a minimum of £5 million in waste annually in a good season. As supermarket requirements vary day to day, growers often need to store harvested fruit for an extra 24-48 hours. Fruit firmness is essential to maintain quality, enabling fruit to withstand such extensions to storage time before transport across the UK and beyond. This is in addition to the 7 days of shelf-life demanded by supermarkets. So, 24 to 48hours extension in shelf life improvement in fruit shelf-life would increase the value of harvested fruit and reduce waste, and will also further enhance the reputation of UK fruit as a high quality product. This research has potentially highlighted some progeny from the mapping population that merit further investigation such as the extraction of RNA, isolation and identifying sequence polymorphisms in named genes implicated in fruit softness. The database of the sequenced genes may help to accelerate the development of potentially new raspberry varieties with extended shelf-life and concomitant reduction in fruit spoilage and waste in the supply chain.

Table 3. Predicted hardness means for the interaction between Day.Cultivar

Cultivar	Day			
	0	2	4	7
Latham	0.3577	0.4526	0.4436	0.4524
MOY	0.7166	0.7684	0.8506	0.5649
R1	0.4742	0.5484	0.4719	0.4818
R13	0.3729	0.4630	0.4522	0.3837
R42	0.7297	0.8587	0.7564	0.5997
R56	0.4430	0.4110	0.4040	0.3956

R61	0.4725	0.4874	0.4446	0.4052
R62	0.4532	0.5526	0.4920	0.4562
R66	0.5054	0.5282	0.5023	0.4261
R73	0.7372	0.8236	0.6941	0.5774
R88	0.4838	0.5287	0.4731	0.4469
R93	0.4713	0.5674	0.4552	0.5421
R102	0.4190	0.4108	0.4283	0.411
R104	0.4883	0.5989	0.5350	0.5193
R114	0.3822	0.4160	0.3774	0.3616
R136	0.3808	0.4181	0.3982	0.3615
R171	0.5650	0.7041	0.6593	0.5119
R184	0.4854	0.6797	0.6283	0.5637
R214	0.3688	0.5688	0.4937	0.3900
R234	0.4596	0.5549	0.4952	0.3807
R241	0.6187	0.6147	0.5901	0.4626
R248	0.3731	0.3863	0.4373	0.3794
R252	0.7450	0.7809	0.7321	0.5734
R254	0.4573	0.4324	0.4860	0.3723

Note: Standard errors of differences (SED)

Average: **0.06890 (>x₂=0.1378)**
 Maximum: 0.08121
 Minimum: 0.05351
 Average variance of differences: 0.004761

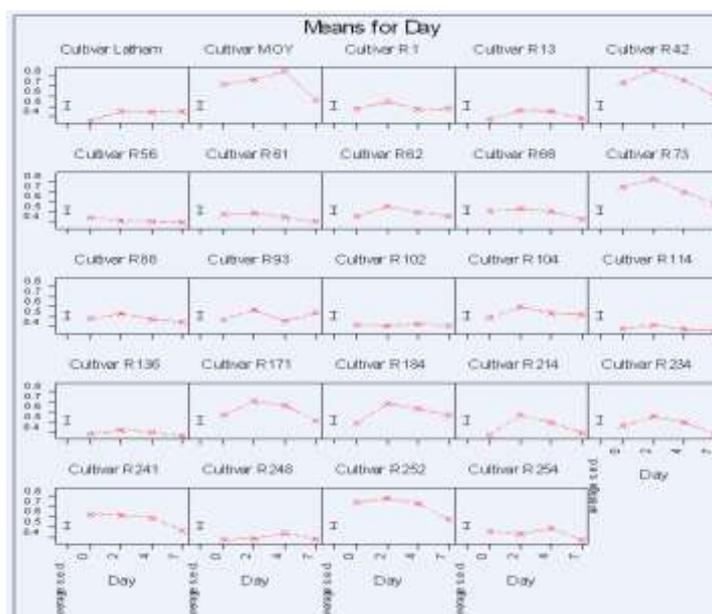


Fig.8. Mean hardness values for all progeny during storage over 0, 2, 4 and 7 days at 4°C.

The results of this research have moved the field of food technology forward by being able to identify not only firmer progeny(s) from the mapping population, but also in the design of a simple and precise methodology for investigating the shelf life properties of soft fruits with the aim of extending the shelf life properties.

IV. Conclusion

Overall, the preliminary storage and shaking (transport) experiments indicated that the QTS-25 Texture Analyzer was suitable for measuring the differences in firmness of raspberry fruit during a time course study and also provided information about the most useful periods of incubation to monitor. Progeny from the mapping population were not significantly different in terms of mean hardness values, the exception being R42, R73 and R252 which were statistically firmer. In addition, no significant differences were observed in mean hardness values between the day of harvest (Day 0) and after 7 days storage except in the cases of clones R73,

R241, and R252, which showed a significant reduction along with Moy after 7 days. This research is hoped to contribute to guidelines as to the most suitable fruit firmness required to maintain high quality fruit from farm to plate thereby reducing spoilage and waste for breeders, growers, processors, supermarkets and the advancement of Food Science and Technology worldwide.

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