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Advances in understanding texture development in apples

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1 Introduction

While apple cultivars were historically appreciated mainly for their taste attributes, such as sweetness, balanced acidity and complex aroma, modern-day consumers also rate fruit texture as important. Texture is, however, a complex concept, and a number of interrelated traits have been identified in consumer tests, including chewiness, cohesiveness, crispness, crunchiness, fibrousness, firmness, hardness, juiciness, mushiness and mealiness (Charles et al., 2018). Sensory panel-based analyses have shown that high values are generally desired for firmness, crispness and juiciness, while mealiness should be as low as possible (Daillant-Spinnler et al., 1996; Vigneau et al., 2014).

Hardness and firmness are often used as synonyms and can be interpreted as the force exerted when biting through a piece of fruit, thereby causing breakage of cell walls as well as affecting cell turgor. By contrast, crispness is often associated with the amount of acoustic energy released when the cell walls are broken. The intracellular juice released by the broken cell walls give

rise to juiciness. Biting into a mealy (floury, starchy) fruit with soft, dry and/or granular fruit flesh causes cells along the middle lamellae of the cell walls to slip and slide without breaking the cell walls. This dampens the sound and lowers the amount of juice released.

Since a large proportion of marketed fruit is stored for several months before it reaches consumers, good texture is important not just at harvest but also following cold storage and during the 1 to 2 weeks at ambient temperatures before the fruit is consumed. Loss of firmness during storage is caused by depolymerization (degradation) of the cell wall middle lamellae polysaccharide structure (Brummell and Harpster, 2001). A dry and mealy texture is related to strong depolymerization of the cell walls, while cultivars with firm and crispy fruit retain cell wall integrity to a greater extent (Longhi et al., 2013).

Storage capability (storability) is extremely important to the grower's economy and must be taken into consideration when deciding what cultivars to grow, and what techniques to apply to growing, harvesting and storing the fruit. Firm fruits tend to tolerate mechanical damage during harvesting and transportation and endure long-term storage as well as attacks from fungal diseases.

There are concerns that some original apple flavors may be lost in newer superfirm cultivars. This chapter will address recent findings related to the concept of fruit texture and how to improve this in new apple cultivars.

2 Anatomy and physiology of fruit texture traits

Fruit anatomy and chemical processes have a significant influence on the apple's textural properties. Although samples of a single apple cultivar ('Golden Delicious') did not show a correlation between size and number of cells in the fruit flesh and variation in fruit flesh texture (Charles et al., 2018), other studies with several different cultivars have indicated a strong relationship between variation in fruit texture and the size and shape of the cells. Smaller intercellular spaces usually co-occur with rounded cells, while fruit flesh with angular cells has larger intercellular spaces. Firm and hard apples tend to have rounded cells and smaller intercellular spaces than softer apples, resulting in more densely packed tissue (Li et al., 2019). Poles et al. (2020) showed that cell size was a more important predictor than cell shape and that smaller cells result in firmer fruit.

Mann et al. (2005) suggested that fruits with a lower number of cells per unit area are crispier than fruits with a higher number and that cell size could be a good predictor of juiciness since bigger cells release more juice than smaller ones, but this has been refuted in other studies. Cell size appeared to have little impact on juiciness, with cell shape being more important; cultivars with

juicy fruit tend to have rounded cells instead of more angular cells (Poles et al., 2020).

Since apples are a climacteric fruit, loss of firmness (fruit softening) is related to the internal level of ethylene, which regulates cell wall degradation (Wakasa et al., 2006; Wei et al., 2010). Fruit ripening-related softening starts during development from fruitlet to the fully ripened stage and is characterized by a pronounced degradation of the pectin in cell walls. This process starts with the loss of the side-chain neutral sugars galactose and arabinose through action of β -galactosidase and α -L-arabinofuranosidase, respectively (Gwanpua et al., 2018). Pectin polysaccharides with a low degree of branching are then demethoxylated by pectin methylesterase. Finally, the demethoxylated pectin chains are attacked by polygalacturonase, resulting in depolymerization of the cell wall pectin.

The softer fruit typical of 'Royal Gala' has been shown to lose more galactose compared to the firmer fruit of 'SciFesh', which has lower β -galactosidase activity and higher cell wall galactan content and thus may better withstand cell wall-modifying enzymes (Ng et al., 2015). Activity of β -galactosidase is higher and pectin degradation is more prominent in 'Fuji', which has crisp fruits that soften markedly during storage, compared to the firm and tough fruits of 'Qinguan', which retain initial firmness during the entire storage period (Yang et al., 2018). Another study showed that galactose and arabinose contribute to the higher hardness of 'Hanfu', while arabinose, egg-box structure and fucosylated xyloglucans improve cell adhesion and thus contribute to the higher crispness of 'Honeycrisp' (Yang et al., 2022).

Generally, firmness decreases during the harvesting period, with a major reduction in conjunction with or, more commonly, just after the climacteric rise in IEC (internal ethylene concentration; Tahir and Nybom 2013). Commercial fruit, especially if intended for long-term storage, is usually harvested just before the rise in IEC. Harvesting the fruit later leads to faster deterioration of overall quality. In an experiment, when 'Golden Delicious' was harvested 1 (T1) or 3 weeks (T2) after optimal harvesting time (T0), the T2 samples especially were less hard and crunchy and more mealy and grainy compared to T0 and T1 samples, according to both instrumental and sensory panel data (Charles et al., 2018).

Even when harvested at the optimal stage, fruit texture changes conspicuously during storage. For germplasm evaluation and selection of breeding materials, fruit texture parameters are therefore often measured both at harvest and after storage. Loss in firmness (difference in firmness between measurements) is sometimes divided by number of weeks in storage to yield 'softening rate' (Nybom et al., 2013). In addition, measurements are sometimes conducted following removal of the fruit from storage in order to assess the shelf-life, i.e. the period when the fruit is exposed to ambient temperatures before purchase and consumption.

The ability to maintain quality throughout a period of cold storage is most likely influenced by differences in ethylene production as well as cell size and cell wall structures. Early-ripening cultivars (summer and early autumn apples) generally have high climacteric respiration and higher levels of ethylene production and therefore mature and soften quickly (Janisiewicz et al., 2008). By contrast, late-ripening cultivars (late autumn and winter apples) have lower respiration and ethylene production rates, they mature more slowly and retain firmness better during storage. In addition, early-ripening cultivars generally have larger cells and larger intercellular spaces compared to late-ripening ones (Johnston et al., 2002).

3 Evaluation of fruit texture parameters

Sensory evaluations of various different textural traits have been carried out on apples, using both mass-testing on hundreds of untrained consumers (Jönsson and Nybom 2007) as well as trained panels comprising 10–30 respondents (Dailliant-Spinnler et al., 1996; Hampson et al., 2000). To untrained consumers, texture can be hard to evaluate since the difference between firmness and crispness is not always fully understood. Evaluations with trained sensory panelists have, however, also yielded variable results (Teh et al., 2020). Sensory crispness is generally moderately correlated with sensory juiciness, and sometimes with hardness, but the set of apple cultivars tested differs widely between studies, as do the training and terminology used by panelists. Moreover, the number of samples that can be assessed by the same taste panel within a restricted time frame is quite small.

Instrumentally derived data are usually less expensive and can be obtained in very large numbers, thereby providing the sample sizes needed for in-depth physiological and genetic studies. Relationships between sensory panel variables and instrumental measurements must, however, be carefully defined (Bejaei et al., 2021). Predictive models using both sensory and instrumental data were shown to explain more than 85% of the variation for hardness and crispness, but accurate models for juiciness and skin toughness were more difficult to achieve (Fig. 1).

Firmness (i.e. hardness) is the most commonly investigated parameter and is easily assessed in the field by a puncture or pressure test administered by a simple handheld penetrometer (e.g. Effegi) using a metal probe, 8–11 mm in diameter with a mildly convex or flat tip. The difference between peeled (most common) and unpeeled samples must be taken into consideration since the skin can contribute around 60% of overall firmness (Grotte et al., 2001; Costa, 2016). For large-scale research data, the probe is often attached to an electronic device that allows linear, constant movement of the crosshead with the probe, e.g. Instron. Frequently, these measurements over-emphasize the

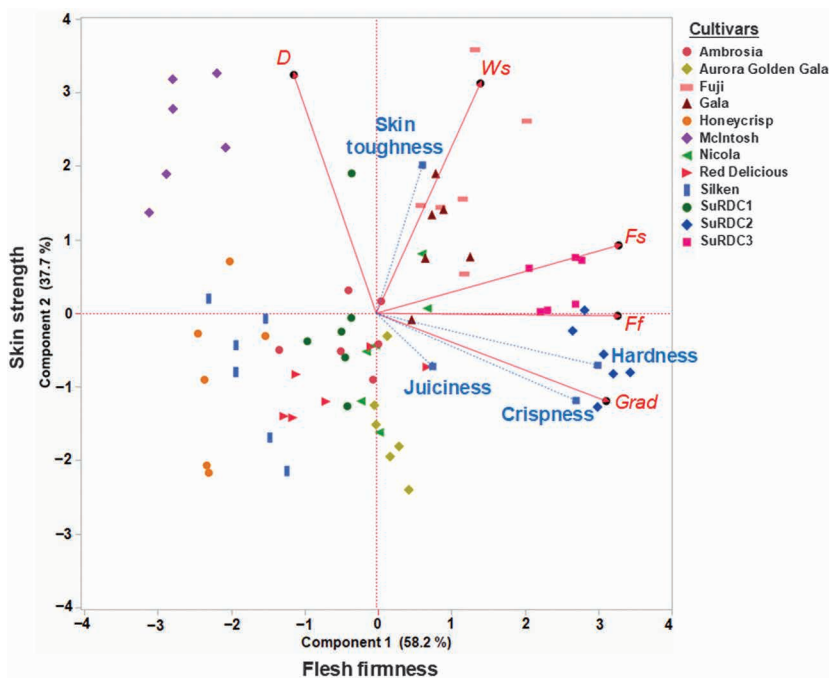


Figure 1 Principal component analysis biplot calculated using standardized mean values of 5 TA.XTplus Texture Analyzer parameters (*Fs*, *Ws*, *Grad*, *D* and *Ff*). Instrumental parameters are identified by red lines. Samples of the 12 apple cultivars/selections are identified with symbols of unique colors and shapes. Sensory attributes (crispness, hardness, juiciness and skin toughness) are identified by blue lines and positioned on this plot using correlation analysis. Source: Reproduced with permission from Bejaei et al. (2021).

influence of the outer cortex of the fruit since the probe does not reach the inner parts.

Since crispness is related to the sound emitted when eating an apple, various acoustic methods have been applied to assess this parameter. The instrumental acoustic-impulse response technique consists of administering a gentle tap on the fruit and measuring the frequency of the sound within the audible spectrum. The obtained readings are strongly associated with the water content and turgor of the fruit. An automated texture analyzer (e.g. TA.XT Texture Analyzer) equipped with an acoustic envelope device measures both mechanical and acoustic parameters on the same sample. Data from this type of instrument has proven to yield superior results compared to measuring the acoustic response from human testers actually biting into the fruit (Piazza and Giovenzana, 2015).

When applied to fruit at harvest, penetrometer-derived estimates of firmness may show little association with acoustic-based estimates of

crispness (Gwanpua et al., 2015; Sadar et al., 2018). However, both methods produce progressively lower values when applied to stored fruit, especially during the first months of storage (Sadar et al., 2018). When applied to shelf-life assessments, acoustics-based values indicated a consistent decrease in crispness as expected, while penetrometer values were more unpredictable (Sadar et al., 2018). The attachment of carboxyl groups arising from pectin degradation can lead to a drier and more rubbery fruit texture (Gwanpua et al., 2015), sometimes producing constant or even higher penetrometer estimates of peel hardness and fruit flesh firmness in stored fruit compared to fruit at harvest (Nybom et al., 2013; Spoor et al., 2019; Butkeviciute et al., 2021).

An informative overview of studies on correlations between instrumental data and sensory panel-based evaluations of various apple texture parameters has been provided by Kim et al. (2022). A strong positive association has usually been found with perceived firmness, as well as with crispness, crunchiness, hardness and juiciness, and a weak negative association with mealiness. Generally, a combination of mechanical and acoustic parameters show significant associations with sensory panel-derived scores for hardness, crispness, juiciness and mealiness when different apple cultivars are investigated (Zdunek et al., 2011; Corollaro et al., 2014; Ting et al., 2015; Charles et al., 2018). When analyzing 86 apple cultivars with a texture analyzer, mechanical impact-based parameters showed high correlation to firmness, while acoustics-based parameters corresponded to crispness as perceived by human senses (Costa et al., 2011, 2012). High crispness always co-occurs with high firmness, whereas apples with low crispness can have any level of firmness (Costa et al., 2011; Ting et al., 2015). In another study, it was shown that the same cultivar may have high values for parameters associated mainly with firmness but low values for parameters associated mainly with crispness (Poles et al., 2020).

An especially large study, based on 11 years of routine fruit quality evaluations with both instrumental and sensory panel data, was carried out in an American apple breeding program (Teh et al., 2020). A Mohr Digi-Test computerized penetrometer, which permits the testing of different layers of fruit, was used to assess hardness (force encountered by the test plunger) and crispness (energy released during the fruit tearing). Instrumental hardness traits significantly correlated with sensory perceptions of hardness, while a lower but still significant correlation was found between instrumental crispness values and the sensory evaluation of crispness, which was influenced also by juiciness. In another study, three different penetrometers (Fruit Texture Analyzer, Mohr Digi-Test-2 and TA.XTplus Texture Analyzer) were applied to the same eight cultivars and associations between the different sets of instrumental data and a set of sensory data were investigated, and models developed for converting data between the different instruments (Bejaei, 2022).

Attaching a friction rig with polypropylene balls to a texture analyzer helped yield data that correlated with various fruit texture parameters (Kim et al., 2022). Friction coefficients were positively correlated with sensory panel-derived scores for crispness and juiciness, and negatively correlated with scores for mealiness and 'rate of melt'. Optical measurements have also been used to assess firmness; time-resolved reflectance spectroscopy in the 580–1064 nm wavelength region showed the potential for discrimination between cultivars (Vanoli et al., 2018a). Yet another option is aquaphotomics based on investigating water-light interactions with NIR (Near Infra-Red) spectrometry (Vanoli et al., 2018b). Hydrolyzation of the pectin apparently affects water structures in the fruit and thus produces water spectral patterns that change according to differences in fruit texture.

4 Influence of growing conditions

Apples are grown in a wide range of climates and experience major differences in terms of quality and quantity of light, temperature, moisture and day length. Fruit from Northern lowland areas (Belgium) had a firmness corresponding to fruit grown at 1000 m in South Tyrol (Sadar et al., 2018), indicating that cultivars developed for colder areas may become too soft when grown in a warmer climate. 'Braeburn' and 'Kanzi' varieties harvested at two different altitudes (300 m and 650 m) and stored in commercial controlled atmosphere (CA) storage for 9 months were shown to differ substantially in firmness (Tijskens et al., 2018). It is likely that lower growing temperatures at higher altitudes reduce the cell division rate during the early growth phase, which results in smaller and firmer apples. Fruit from the same cultivar but grown at higher altitudes similarly had higher initial firmness compared to fruit grown at lower altitudes (Sadar et al., 2018). In another set of analyses, fruit from lower altitudes were juicier, crunchier and sweeter compared to high-altitude samples, which were described as more mealy, sour and astringent by a sensory panel (Charles et al., 2018). Texture performance, soluble solids content and titratable acidity corroborated this sensory description. Moreover, anatomical data showed that fruit from lower altitudes had a larger volume, a higher number of cells and a higher percentage of intercellular spaces.

Commonly applied production systems, i.e. organic, conventional and integrated, provide different environmental conditions for fruit trees and may consequently affect fruit texture, even on a local scale. In a set of studies in Washington state in the United States, organically grown apples were shown to be significantly firmer than same-sized fruit in other production systems (Reganold et al., 2001; Peck et al., 2006). Even small changes in irrigation, mulching, pruning and fertilization have been shown to affect fruit firmness at harvest and/or after storage and/or after 1 to 2 weeks of shelf-life testing

(Mpelasoka et al., 2001; Tahir and Gustavsson, 2010; Tahir et al., 2015b). Differences in crop load can also affect firmness, likely due to variations in the amount of available photoassimilates per fruit (Serra et al., 2016; Tijssens et al., 2018).

5 Fruit texture and storability

The effects of different storage conditions on fruit quality, including nutritional value, taste and texture (especially firmness) have been studied in many apple cultivars. For early-ripening cultivars, a desirable texture, i.e. crispy and medium firm, is needed at harvest since the fruit is consumed within a few weeks. Medium- and late-ripening cultivars need to retain a desirable texture for much longer since the fruit is stored for several months and even up to a year in modern facilities with CA.

Despite cultivar-dependent optimization of storage conditions, firmness, crispness and juiciness usually decrease during cold storage. Treatment of stored apples with 1-methylcyclopropene (1-MCP) is an efficient way to delay fruit softening and is now used in many parts of the world, although not for organically grown fruit. Recently, 1-MCP treated fruit of 'Hwangok' and 'Picnic' varieties, stored up to 6 months at 0°C, were shown to maintain firmness and exhibit lower internal ethylene concentrations compared with untreated fruit (Win et al., 2021). Analyses of both treated and untreated samples suggest that 1-MCP maintained cell wall pectin and delayed softening by reducing solubilization of polyuronides and neutral sugars and limiting cell wall hydrolysis due to ethylene-dependent processes.

Some of the inherent differences in storability can be explained by inter-cultivar differences in texture dynamics (Costa et al., 2012). Three major trends have been identified in 83 cultivars following a comparison of texture analyzer data taken both at harvest and after 2 months of cold storage. Cultivars such as 'Golden Delicious' and other older varieties showed a general decrease in both mechanical and acoustic profiles during storage. By contrast, cultivars like 'Maigold' remained stable throughout the entire storage period. Finally, a set of cultivars including 'Fuji' showed a slight increase, especially in acoustic parameters. In general, apples lose density and increase in volume during storage, resulting in higher intercellular air fraction. A high acoustic response following storage can perhaps be explained by a combination of a high air fraction, high turgor and high integrity of the middle cell wall lamellae.

Ripening period (number of days after flowering to optimum harvest date) was positively correlated with firmness at harvest and negatively correlated with softening rate in 127 Swedish-grown cultivars (Nybom et al., 2013). Corresponding associations between ripening period, firmness at harvest and softening rate have also been reported in other studies (Tahir et al., 2015a;

Migicovsky et al., 2021). In addition, polyploid cultivars have displayed significantly greater firmness at harvest compared to diploid cultivars but similar softening rates (Nybom et al., 2013). Interestingly, fruits with a lower number of seeds (typical also for polyploid apples) have higher firmness than fruit with many seeds, possibly due to a variation in ethylene production (Buccheri and Di Vaio, 2005).

6 Fruit texture and fungal diseases

Variation in storability is closely associated with resistance to fungal storage diseases (Blažek et al., 2007). This factor becomes especially important in integrated and organic production where chemical fungicides are restricted or even prohibited. The resulting lack of protection enhances susceptibility to these fungi, and a substantial amount of fruit is destroyed each year. Most of this damage is caused by ascomycete fungi, known as storage rots, which attack apples both in the orchard and during cold storage (see review in Nybom et al., 2020). Symptoms are first visible as lesions on the fruit epidermis and can proceed to rotting of the entire fruit. Research data on susceptibility to different rots is based mainly on experimental inoculations, usually involving the transfer of conidiospores into apple flesh with a micropipette (wound-inoculation). The diameter of the resulting lesion is measured after several weeks of cold storage and used as a measure of susceptibility.

Some of the most significant storage rots in apples belong to two biotrophic or hemibiotrophic genera also known as latent infection pathogens, namely *Neofabraea* (= *Pezizula*) and *Colletotrichum*. Apple cultivars assessed from natural infection to be resistant to *Neofabraea* had somewhat firmer flesh on average than susceptible cultivars (Blažek et al., 2007) but no correlation was found with firmness when dipping fruit of 18 cultivars in a spore suspension of *Colletotrichum acutatum* (Biggs and Miller, 2001). A correlation between the amount of softening during storage and susceptibility was found after wound-inoculating with *Colletotrichum gloeosporioides* in a set of 36 early-ripening cultivars, although not for 34 late-ripening ones (Ahmadi-Afzadi et al., 2013). Neither set of cultivars showed any impact on firmness at harvest. Although fruit texture may play a role, tolerance to these fungi depends mainly on the number of lenticels (major entry points) and the thickness of the cuticular layer of the fruit, as well as various resistance genes, although none has as yet been identified.

Some mainly necrotrophic fungal species are known as 'wound pathogens' and include, among others, *Penicillium expansum*, *Botrytis cinerea*, *Monilinia fructigena* and *Monilinia laxa*. One QTL (quantitative trait loci) for relatively strong resistance toward the most well-researched of these fungi, namely *P. expansum* which causes blue mold, has been identified on linkage group (LG) 3 in the apple's

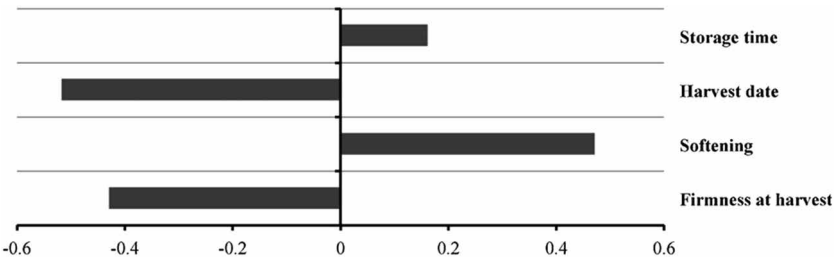


Figure 2 Weighted regression coefficient indicating the importance of four independent variables: storage time (number of weeks in storage), harvest date (ripening time), softening and firmness at harvest in explaining variation in size of lesions produced after artificial inoculation of 92 Swedish-grown apple cultivars with *P. Expansum*. Source: Reproduced with permission from Ahmadi-Afzadi et al. (2013).

wild relative *Malus sieversii*, while another QTL on LG 10, co-occurring with QTLs for firmness and ripening, provided lower-level resistance and was presumably inherited from ‘Royal Gala’ (Norelli et al., 2017). More research is required to unravel the genetic structure among these QTLs and their interdependence.

Animal- or man-made wounds in the fruit constitute major entry points for wound pathogens. Several experiments point to the role of fruit flesh texture in terms of tolerance to these fungi, reflecting the ease with which an infection point can lead to a large lesion. Regression analyses with *P. expansum* lesion diameter as a dependent variable demonstrated a negative effect of fruit firmness at harvest in 46 late-ripening cultivars but not in 46 early-ripening ones (Ahmadi-Afzadi et al., 2013). The amount of fruit softening during storage had a positive effect on lesion diameter in late-ripening cultivars but not in early-ripening (Fig. 2). In a follow-up study on 81 apple cultivars, lesion diameter was again negatively associated with fruit firmness at harvest and positively associated with the amount of fruit softening during storage (Tahir et al., 2015a). Similar results were reported in a smaller study by Costa et al. (2005). A negative correlation was also found between fruit firmness and lesion diameter in a study of Iranian cultivars (Naeem-Abadi et al., 2014). The relationship between firmness and lesion decay reported here is likely associated with the ability of cell walls to withstand attacks from pectolytic enzymes of the fungus.

Susceptibility to *P. expansum*, as well as to another wound-infecting species, *B. cinerea*, is apparently associated with ripening time; later cultivars appear to be more tolerant (Davey et al., 2007; Ahmadi-Afzadi et al., 2013; Tahir et al., 2015a). One reason could be that late-ripening apple cultivars generally have a lower ethylene-regulated climacteric burst and therefore higher fruit firmness.

7 Genetic determination of texture

Increasingly accurate genetic maps for apples are now available, with candidate genes that have an impact on ethylene production. In 2012, numerous QTLs

Table 1 List of apple texture loci mentioned in this chapter, their linkage groups, major effects reported and references

Name	LG	Effect	Ref.
<i>Md-Exp7</i>	1	cell wall breakdown: softening	Costa et al., 2008
QTL mealiness	1	mealiness	Kunihisa et al., 2016
<i>Md-ERF4</i>	3	ethylene regulation: firmness and softening	Hu et al., 2020
<i>Md-ERF3</i>	3	ethylene regulation: softening	Wu et al., 2021
<i>NAC18.1</i>	3	regulates ripening: firmness and softening	Migicovsky et al., 2021
<i>Md-ACO1</i>	10	ethylene regulation: firmness and softening	Costa et al., 2005
<i>Md-PG1</i>	10	cell wall breakdown: firmness/ crispness, softening, juiciness	Longhi et al., 2013; Poles et al., 2020
QTL watercore	14	watercore	Kunihisa et al., 2016
<i>Md-ACS1</i>	15	ethylene regulation: firmness and softening	Oraguzie et al., 2004
<i>Md-ACS3a</i>	15	ethylene regulation: softening	Wang et al., 2009
<i>Md-ERF118</i>	16	ethylene regulation: softening	Wu et al., 2021
<i>Ma</i>	16	malic acid regulation: acidity but also firmness	Ru et al., 2021
<i>Md-XTH</i>	16	cellulose/hemicellulose network: crispness retention	Chang and Tong, 2020
<i>Md-β-Gal</i>	?	ethylene regulation: fruit ripening	Farneti et al., 2021

were identified as having a significant impact on fruit texture (firmness, hardness, crispness, juiciness, sponginess, compression and slow breakdown) and correlated to 10 of the 17 LGs in apple (review in Maronedze and Thomas, 2013). Associations between allelic configuration in various candidate genes and fruit texture parameters have been shown, as these genes also co-occur with several of the previously identified QTLs (Costa et al., 2010; Longhi et al., 2012) (Table 1).

Md-ACS1 (1-aminocyclopropane-1-carboxylate synthase) has a significant influence on fruit firmness and rate of softening during storage (Oraguzie et al., 2004). Two alleles were identified: allele 2 is associated with reduced ethylene production and thus firmer fruit and slower softening while allele 1 results in normal ethylene production. The three alleles found in *Md-ACS3a* also appear to be closely associated with ethylene production and shelf-life in apples (Wang et al., 2009). Another gene that can affect fruit firmness is the biallelic *Md-ACO1* (1-aminocyclopropane-1-carboxylate oxidase; Costa et al., 2005, 2010; Zhu and Barritt, 2008). The *Md-ACS1* and *Md-ACO1* genes have been mapped to LG 15 and LG 10, respectively (Costa et al., 2005; Chagné

et al., 2019) and DNA markers have been applied to the screening of several large sets of apple cultivars (Sunako et al., 1999; Harada et al., 2000; Oraguzie et al., 2004, 2007; Nybom et al., 2008; Zhu and Barritt, 2008).

Interestingly, QTLs for different texture traits, as well as related traits such as ripening period, are often located in the same regions, suggesting that genetic variation in one or several interrelated genes may affect numerous traits simultaneously. Some of the steps in the ethylene-driven regulatory network determining fruit firmness and softening were revealed recently in a gene expression study following the detection of a mutation in the *ETHYLENE RESPONSE FACTOR (ERF4)* gene on LG 3 (Hu et al., 2020).

Much research has also focused on the cell wall and its degradation during fruit ripening. The biallelic endopolygalacturonase gene *Md-PG1* has been mapped to LG 10, just 37 cm from *Md-ACO1* (Costa et al., 2010; Longhi et al., 2013; Chagné et al., 2019). Allelic variation in *Md-PG1* can explain up to 40% of the phenotyped variation in firmness and texture, and DNA markers are now being exploited in several apple breeding programs around the world. Genetic configuration can, together with fruit flesh anatomy, explain some of the phenotypic variations in other texture traits; homozygosity for the low-softening allele in *Md-PG1*, in combination with a high fraction of rounded cells in the fruit flesh, was found to be strongly associated with high levels of juiciness in a set of 14 apple cultivars and selections (Poles et al., 2020). Heterozygous cultivars and/or cultivars with more angular cell shapes showed lower levels of juiciness.

Expansin enzymes affect depolymerization of different polysaccharides in the cell walls during fruit softening and may therefore also be of interest. An expansin homolog, *Md-Exp7*, was mapped on LG 1, and a functional marker was developed by Costa et al. (2008). However, screening a large set of genotypes has shown that there are multiple alleles, and their effects are difficult to interpret (Nybom et al., 2013).

In contrast to the instrumentally derived phenotypic data generally used in research on fruit texture genetics, Amyotte et al. (2017) performed a GWAS (genome-wide association study) based on descriptive data provided by a trained sensory panel for a collection of 85 apple cultivars. Genomic associations were recorded for several traits including crispness, juiciness and mealiness. Some of the detected QTLs resided in genomic regions not implicated in previous studies and may therefore target different genes.

The availability of large numbers of mapped single nucleotide polymorphisms (SNPs) has simplified the detection of QTLs and determination of minor gene impact on texture traits. Firmness, which can be scored rapidly in large materials, has now been investigated in traditional biparental mapping populations as well as in pedigree-based analyses (PBA) and GWAS. In one study, a GWAS was performed on a collection of 233 apple accessions

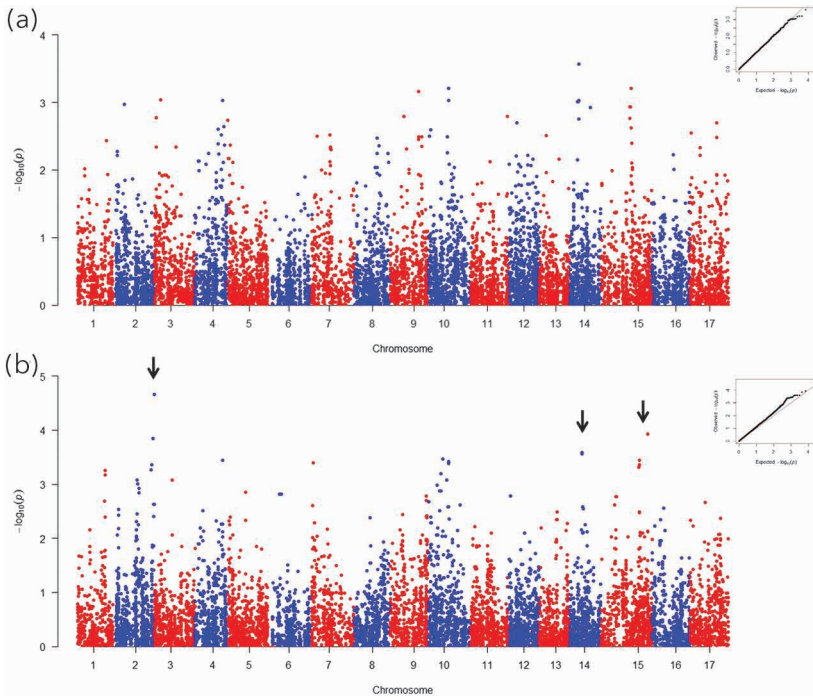


Figure 3 Manhattan plot illustrating the association between SNP markers and two texture subtraits assessed with a high-resolution texture analyzer, maximum force (a) and number of acoustic peaks (b), computed in a panel of 233 apple accessions. The x- and y-axes report the number of chromosomes and the $-\log_{10}(P\text{-value})$, respectively. For both panels, the Q-Q plot is also reported. Source: Reproduced with permission from Di Guardo et al. (2017).

together with a PBA of six full-sib pedigreed families (Di Guardo et al., 2017). DNA polymorphisms were determined with a 20K SNP array and fruit texture assessments with a sophisticated high-resolution texture analyzer. QTLs were identified on LG 10 (especially mechanical properties) and LG 2 and 14 (acoustic response properties) (Fig. 3).

In another study, more than 8000 SNPs were used to develop a model for genomic selection in apples (Roth et al., 2020). A set of 537 genotypes were first phenotyped for mechanical and acoustic parameters, and a training set of 259 genotypes with high phenotypic variability was chosen. A principal components analysis showed that the two major components correspond to firmness and crispness, respectively, with firmness being the most accurately estimated. Although most attention has focused on firmness and crispness, QTLs for other traits have also been detected, e.g. one QTL for mealiness in LG 1 in a set of 'Fuji'-related accessions (Kunihisa et al., 2016).

Recently, transcriptomic analyses have revealed additional genes involved in ethylene production and cell wall structure and degradation, which are differentially expressed in genotypes with opposing fruit texture traits. Several candidate genes for crispness retention during storage were revealed in a study of 'Honeycrisp' and some of its offspring, including *Md-XTH*, which regulates the cell wall-modifying enzyme xyloglucan endotransglucosylase/hydrolase (Chang and Tong, 2020). Another interesting gene is *Md-β-Gal2*, which determines β-galactosidase levels and appears to be associated with fruit ripening (Yang et al., 2018).

In addition to genes directly involved in ethylene biosynthesis and pectin degradation, several transcription factors such as ethylene response factors (*ERF*) which bind to, for example, the *Md-ACS1* promoter, can have a significant impact on fruit texture parameters. Wu et al. (2021) used bulked segregant analysis and RNA-seq to identify 62 QTLs in a set of 2664 apple seedlings. A total of 56 candidate genes were analyzed and shown to predict 55% and 60% of the variation in retention of firmness and crispness, respectively. Functional validation of these candidates provided evidence that small deletions in the *Md-ERF3* promoter (LG 3) and in the *Md-ERF118* promoter (LG 16) reduce the retention of firmness and crispness following storage through their impact on several important genes.

Another transcription factor gene, *NAC18.1*, affected both firmness at harvest and softening during storage in 800 apple accessions (Migicovsky et al., 2021). This gene, located on LG 3, outperformed the commonly screened *Md-ACS1*, *Md-ACO1* and *Md-PG1* in terms of predicting firmness at harvest and after storage. Softening rate was, however, more accurately predicted with *Md-PG1*. The *NAC18.1* gene encodes a protein that is orthologous to a transcription factor (*NON-RIPENING*), which regulates ripening in tomatoes. It can be presumed that it acts by modulating the ripening process in apples as well.

8 Association between texture and aroma

Apple flavor can be defined as a combination of taste (perception in the mouth) and aroma (mainly perceived by olfactory receptors). Taste is often estimated as the amount of sugars and organic acids, and the ratio between these compounds. Aroma is dependent on a complex cultivar-specific mixture of many volatile organic compounds (VOCs), estimated to comprise at least 300 different molecules in apples (Ulrich and Dunemann, 2012). The delimitation between these concepts is not exact since fruity esters can contribute to the taste of sweetness (Ting et al., 2015; Aprea et al., 2017).

The aroma profile changes as the fruit ripens, starting with a predominance of aldehydes, followed by an increase in alcohols and ending with a predominance of ester compounds at maturity (Fellman et al., 2000).

Consequently, ripening-related processes play an important role in determining the type and amount of aroma in any given fruit. Aroma is also affected by environmental factors, as shown in a set of 11 cultivars grown in two different locations in northern Italy (Chitarrini et al., 2020). Some currently applied storage methods, as well as treatment with 1-MCP, can alter the VOCs as well as the sensory perception of the fruit in a negative way (Ting et al., 2015).

Unfortunately, cultivars with favorable fruit texture (firm and crispy) generally have a comparatively restricted aromatic profile, while highly aromatic cultivars tend to be softer. Fruits of 162 cultivars grown in northern Italy were analyzed for texture and aroma as well as VOC phenotyping following 2 months of cold storage (Farneti et al., 2017). An SNP-based GWAS indicated that *Md-PG1* is not only responsible for fruit texture but also has a direct effect on the quantitative emission of volatile compounds. A gene expression analysis similarly showed that *Md-PG1*, together with four other texture genes (*Md-ACS1*, *Md-ACS3*, *Md-β-Gal* and *Md-ACO1*), was up-regulated toward the end of a 2-month storage period of the high-ethylene 'Golden Delicious' but remained at a basal level in the almost non-climacteric 'Fuji'. However, a burst of ethylene is necessary to stimulate the activation of genes fundamental to the production of volatile compounds (Busatto et al., 2016).

The physiological disorder watercore is caused by the rapid breakdown of cell walls and fluid filling up the intercellular spaces. The commonly occurring watercore fruits of several old European apple cultivars used to be appreciated for their enhanced sweetness and juiciness but are now avoided due to flesh break-down and browning during long-term storage. Watercore is, however, still desirable in some Asian countries where affected fruit is marketed as being sweeter and more aromatic. Apparently, ethyl ester synthesis is enhanced under hypoxic conditions within watercored tissues, resulting in a distinctive, fermented flavor (Tanaka et al., 2020). While many watercore-prone cultivars rapidly become soft and mealy, other cultivars like 'Fuji' remain firm in spite of developing watercore. A QTL for watercore development in 'Fuji' and its relatives has been detected in LG 14 (Kunihisa et al., 2016). Although watercore and mealiness often occur together, many newly developed watercore-susceptible lines derived from 'Fuji' lack this mealiness and can have both excellent flavor and texture (Tanaka et al., 2020).

9 Selection/breeding achievements

Selective sweeps of several traits have helped shape modern apples, as deduced from genetic sequences of modern apple cultivars and progenitor species such as *Malus sieversii* and *M. silvestris* (Duan et al., 2017). One region on LG 16 with several polygalacturonases, one region on LG 17 with cellulose synthase genes, and another on LG 12 with pectin esterases have experienced

intensive human selection for fruit firmness. Further examination of these regions may assist in detecting genes for utilization in modern apple breeding.

A more recent development has involved the selection of particular genes, although the heritage of the targeted traits was unknown. The frequency of the firmness-promoting allele 2 of *Md-ACS1* has thus increased substantially, from below 20% in older cultivars (developed before 1800) to more than 50% in recently registered cultivars (from 1960 onward), indicating that this allele has been favored by selection for improved fruit quality in modern apple breeding programs (Nybom et al., 2008). Allele frequencies for numerous genes were similarly quantified in ancestral accessions and progenies included in the apple REFPOP (Jung et al., 2022). Compared to ancestral accessions, the allele with an increasing effect on phenotype had a higher frequency in the progeny for fruit firmness as well as for some other traits (later harvest date, increased flowering intensity, titratable acidity and trunk increment).

To date, thousands of apple cultivars have been selected and propagated around the world, and prominent gene flow across Europe has been revealed (Urrestarazu et al., 2016). Commercial production is, however, dominated by a very small number of genotypes, most of which are closely related (Muranty et al., 2020), and access to highly variable germplasm is therefore crucial for breeding purposes. Moreover, the need for carefully defined protocols for sensory and instrumental data collection has been highlighted in large phenotyping projects involving unreplicated genotypes planted at different locations (Schmitz et al., 2013).

New cultivars are generally developed by crossing suitable parental genotypes followed by screening the resulting seedlings in the field and evaluating propagated trees in test orchards. Large-scale screenings of textural traits undertaken in apple progenies have shown there is ample room for further improvement. Histological phenotyping revealed substantial variation in cell size distribution in a biparental apple progeny while sensory and instrumental analyses revealed variation in parameters, including firmness, crispness, graininess and juiciness (Gálvez-López et al., 2011a), as well as chemical composition and structure of cell wall polysaccharides (Gálvez-López et al., 2011b).

For breeding and selection purposes, the heritability of the different fruit texture parameters in a relevant plant material is crucial. Several estimates around 50% have been reported for firmness but crispness is usually considerably lower, e.g. 23% in a study on apple seedlings from 25 biparental crosses (Ru et al., 2021). Many estimations have, however, been based on mapping populations where very different genotypes are contrasted and may not be fully valid for apple breeding programs focusing on elite genotypes.

Recently, MAB (marker-assisted breeding) and MAS (marker-assisted selection) have been implemented in some apple breeding programs, both for major genes and for QTLs. The RosBREED SNP Consortium OpenArray v.1.0

assay with 128 SNPs linked to loci that determine fruit quality and pest and disease resistance was developed for MAS in breeding programs (Chagné et al., 2019). This array includes 15 SNPs across five fruit texture trait loci (*Md-PG1* and *Md-ACO1* on LG 10, *Md-ACS1* and QTLs derived from 'Braeburn' on LG 15 and LG 16). Three *Md-PG1* SNPs and one SNP for *Md-ACS1* proved to be significantly associated with BLUP (Best Linear Unbiased Prediction) values for firmness and crispness of advanced selections and commercial cultivars and were further validated using average phenotypes in advanced selection and validation families.

DNA markers are often used to screen putative parents. The cost-effectiveness of screening the seedlings depends on the percentage of seedlings that can be discarded at an early stage (Wannemuehler et al., 2019), which in turn depends on the number and efficiency of the DNA markers scored. When 127 Swedish-grown apple cultivars were screened for allelic composition in four fruit texture genes, alleles previously described as having good texture were associated with significantly lower softening for *Md-ACS1* and *Md-PG1*, but the opposite was noted for *Md-EXP7*, while results were insignificant for *Md-ACO1* (Nybom et al., 2013). These markers accounted for 15% of the observed variation in initial firmness and 18% for softening rate. The inclusion of ripening period, storage time (i.e. 6 or 12 weeks) and initial firmness into the model increased the predictability of softening rate to 38%.

In another study based on 321 seedlings in a US breeding program, genotyping with *Md-ACS1*, *Md-ACO1*, *Md-PG1* and *Md-Ma* (mainly affecting acidity) produced a 16% increase in additive variance for apple crispness and 17% for firmness (Ru et al., 2021). Of the different loci tested, only *Md-PG1* was significant for both firmness and crispness while *Md-Ma* was significant for firmness but still explained much less than *Md-PG1*. Similarly, only *Md-PG1*, together with the newly detected *NAC18.1*, had any predictive power in a set of 800 cultivars phenotyped for firmness at harvest and after storage and softening (Migicovsky et al., 2021). Even when the ripening period (harvest date) was entered into the model, variation in *NAC18.1* could only predict 18% of the variation in firmness. More powerful markers are therefore needed for large-scale, cost-effective applications in breeding programs.

10 Conclusion and future trends

Of several described texture traits, firmness, crispness, juiciness and mealiness are the most studied, with data obtained from both instrumental and sensory analyses. Fruit anatomy (cell shape and size) and physiology (ethylene production and enzymatic degradation of cell walls) have a major impact on fruit texture, as do environmental factors such as altitude and orchard management

practices. Fruit texture is an important determinant for storability of different cultivars as well as their tolerance to fungal storage diseases, but unfortunately there appears to be a negative relationship between flavor and firmness.

Several major genes and QTLs have been identified as having a significant impact on firmness, and DNA markers have been developed for use in plant breeding. More efficient markers for firmness as well as for other texture traits are, however, needed for seedling selection with a high predictability of good fruit texture.

In the future, large genotyping arrays, such as the 20K and 480K SNP developed for apple (Bianco et al., 2014, 2016) and large-scale genotyping-by-sequencing, together with improved data analyses such as GWAS and GAP (Genomics-Assisted Prediction) and transcriptomics will improve our understanding of the intricate networks that regulate fruit texture traits. Development of DNA assays with carefully selected markers for key genes will become increasingly important in applied apple breeding programs, provided that cost-effective levels of selection success can be achieved.

Lack of high-quality phenotyping data has been identified as a critical factor. In addition to exact and informative measurements, the potential to assess genotype-environment interaction by measuring the same trait over several years – and preferably on trees growing in different orchards – would be very helpful. Recently, large field collections have been implemented, such as REFPOP where the same apple genotype is planted at six different locations (Jung et al., 2020). Although data is not yet available for all locations, close co-localization between markers for harvest date and fruit firmness has already been shown on LG 16 while firmness and several other traits co-occurred with firmness on LG 3 (Jung et al. 2022).

11 Where to look for further information

11.1 Further reading

Bejaei et al. (2021) present a recent analysis of relationships between sensory and instrumental texture trait data.

Chagné et al. (2019) describe the development and validation of a SNP array for MAS.

Musacchi and Serra (2018) provide a review of pre-harvest factors that have an impact on apple fruit quality. Part of this overview is dedicated to fruit texture but it should also be read for insights into other aspects of fruit quality, many of which are interrelated with texture.

Nybom et al. (2020) describe the impact of apple fruit ripening, texture and chemical contents on genetically determined susceptibility to storage rots.

Wannemuehler et al. (2019) analyze the cost-effectiveness of applying MAB and MAS in apple breeding programs.

Wu et al. (2021) provide a detailed analysis of the regulatory network involved in retention of firmness and crispness during storage.

11.2 Key conferences

EUCARPIA (European Association for Research on Plant Breeding), Section Fruit, organizes conferences every 3–4 years that are relevant to fruit breeding and genetics.

ISHS (International Society for Horticultural Science) organizes a world congress every 4 years, as well as symposia on narrower topics such as biotechnology and molecular breeding, fruit production and post harvest.

RGC (International Rosaceae Genomics Conference) is organized every 2 years and coordinated by the Rosaceae International Genomics Initiative.

11.3 Major research centers and international projects

Multi-partner projects, such as the EU-funded FP7 Fruitbreedomics (2010–2015) and the USDA-SCRI funded RosBREED 1 and RosBREED 2 projects (2010–2018) (www.rosbreed.org) have played a large role in developing international research on apple genetics. Both have resulted in several spin-off projects, emphasizing the need for international cooperation.

In addition to the numerous European and American research facilities involved in the abovementioned projects, valuable research is carried out by Summerland Research and Development Centre and Vineland Research and Innovation Centre in Canada, The New Zealand Institute for Plant and Food Research, and China Agricultural University in Beijing.

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