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Texture and other fruit quality parameters profiling for sweet cherry and berries breeding

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Padova, 27th September 2018

Lara Giongo

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Riassunto

Il miglioramento per la qualità del frutto dei piccoli frutti, in particolare per tratti altamente soggettivi quali la tessitura della polpa, metaboliti secondari ed aroma è allo stato dell'arte ancora una sfida. Sinora, il miglioramento genetico per texture ed aroma ha sfruttato molto poco metodologie assistite, la MAS per esempio, ed è invece molto dipeso da scelte soggettive dei breeders, in particolare in fasi di selezione molto precoci.

L'obiettivo di questa ricerca è stato quello di sviluppare metodologie validate per le diverse classi di piccoli frutti e ciliegia, che possano oggettivare un tratto complesso e commercialmente molto importante quale la tessitura.

Per le diverse specie esaminate si è applicato un piano sperimentale molto simile. Il primo obiettivo è stato riuscire a capire il grado di variabilità presente nei rispettivi germoplasmi a disposizione, validando, in un ampio numero di cultivars, i parametri descrittivi della tessitura specie-specifici.

Il secondo obiettivo è stato capire quali modifiche la tessitura subisce in due momenti chiave della filiera produttiva e di consumo: al momento della raccolta e dopo conservazione. Per quest'ultimo aspetto si sono scelte le condizioni standard di postraccolta (2°C e umidità relativa tra 85-95% in assenza di atmosfera controllata per sei settimane per mirtillo, 8 giorni per fragola, 3 e 7 giorni per lampone e 7-14 giorni per ciliegio) per comprendere la dinamica della tessitura ed altri tratti correlati con la qualità.

L'analisi delle componenti principali (PCA) sulle diverse proprietà meccaniche, ha così permesso di ottenere una chiara separazione delle varietà di mirtillo (*Vaccinium*) valutate, sia in fase di sviluppo del frutto, a maturazione ed in postraccolta. L'effetto dello stadio valutato e le differenze tra genotipi sono risultati significativamente diversi per i parametri che contribuiscono a definire il profilo di tessitura della specie e di altri tratti qualitativi.

Lo sviluppo di queste metodologie è strettamente connesso con l'anatomia del frutto: tra i frutti considerati, il lampone, ad esempio, presenta, tra tutte, l'anatomia più complessa, essendo un aggregato di drupeole. In questo caso si è quindi scelto un approccio di analisi di tessitura tramite penetrazione ed in parallelo di compressione. Lo studio ha rivelato un'alta variabilità della tessitura all'interno del pool di 29 varietà di lampone valutate. Anche in questo caso, i pattern di

profili di tessitura sono risultati essere significativamente dipendenti sia dal genotipo sia dal momento di analisi (maturazione e/o post-raccolta). Le due metodologie utilizzate hanno inoltre permesso di ottenere profili complementari di decomposizione della texture e i 22 parametri meccanici e morfologici utilizzati hanno permesso di individuare relazioni significative tra tratti che contribuiscono alla qualità del frutto.

Per fragola, i profili di tessitura sono stati determinati su un doppio ciclo di produzione, per 87 diversi genotipi, includendo nel disegno sperimentale sia varietà unifere sia rifiorenti, al momento della raccolta ed in post-raccolta. Anche in questo caso si sono rilevate differenze significative dei profili di tessitura in fase di maturazione del frutto e dopo conservazione, dimostrando anche per questa coltura un grande potenziale nell'utilizzo di queste metodologie per comprendere meglio le diverse componenti di qualità del frutto nei diversi momenti di consumo.

La tessitura della ciliegia (*Prunus avium* L.) è stata valutata in un germoplasma di 36 cultivars. Oltre a differenze al momento della raccolta ed in post-.raccolta, in ciliegio si sono valutati gli effetti sui diversi profili in relazione all'anno di valutazione e dal diverso portinnesto, testato su innesti di due cultivars commerciali (Kordia e Regina).

Per le diverse specie sono stati sviluppati, sulla base dei parametri di tessitura scelti, specifici Indici di Conservazione, che hanno permesso un ulteriore identificazione dell'attitudine di ciascuna varietà alla conservazione.

La validazione dei profili di tessitura e la loro associazione ad altri tratti relativi alla qualità del frutto nei piccoli frutti ed in ciliegio così ottenute mettono definitivamente in risalto che questi tratti sono ancora molto poco sfruttati nel miglioramento genetico, e che c'è quindi un ampio spazio di utilizzo dei parametri come biomarcatori. Il loro utilizzo consente non solo di accelerare i processi di selezione varietale per questi tratti specifici attraverso un'appropriata identificazione, ma possono anche essere validi strumenti nell'intera industria produttiva, quale ausilio nella cernita del prodotto o nella segmentazione di mercato in base a specifiche caratteristiche di tessitura.

Abstract

Breeding for berries quality, and in particular for highly subjective goals like texture, secondary metabolites and flavour is challenging. Moreover, breeding both for texture and aroma has occurred mainly without assisted methodologies but mostly by subjective chance, in particular in early selection phases.

The aim of this work was to obtain high-throughput quality profiles of different berries with regard to a complex and commercially important quality trait like texture.

For the different crops, a similar experimental design was applied. The first aim of the different experiments was to unravel the widest variability for the different traits of interest to determine fruit quality within the respective germplasm. The second aim was to proof the differences present at the two cardinal time-points for the production and commercial pipeline: at harvest and after storage. More precisely storage conditions were: 2°C and relative humidity of 85-95% for six weeks at normal atmosphere conditions for blueberry, 8 days for strawberry, 3 and 7 for raspberry and 7-14 days for cherry, in order to monitor the dynamics of the different quality traits for each genotype.

Principal component analysis based on fruit textural proprieties, allowed for blueberry a distinct separation of the 46 *Vaccinium* cultivars evaluated, revealed a clear separation of the four harvest ripening stages. As expected, storage also highlighted textural differences among cultivars that were magnified compared to ripening. The effect of ripening stage and genetic differences on the blueberry texture profiles and other fruit quality related traits were significantly high.

The development of the texture raspberry methodology is highly related to fruit anatomy, which is more complex in raspberry than in the other berries. A parallel approach of penetration and compression on a double bite cycle measurements was thus chosen. A high variation was explained among 29 raspberry cultivars tested in this study. Differences among genotypes were observed at all ripening stages, showing a significant cultivar dependent pattern at harvest and after storage. The two methodologies allowed to complimentary profile raspberry texture and a clear relationship among 22 texture mechanical parameters and morphological quality traits was elucidated.

For strawberry, the texture profiling was done on a double cycle production for different genotypes, both including in the experimental setting junebearing and everbearing. 87 genotypes were profiled at harvest and post-harvest.

The strawberry texture variation in the genetic pool analysed was explained by changes of different parameters. The development of the fruit was also investigated for texture, morphological and metabolic traits. The results demonstrated the potential of using species specific methodologies towards the comprehensive study of strawberry fruit quality attributes during harvest and storage.

The texture trait of sweet cherry (*Prunus avium* L.) was investigated on 36 cultivars. Changes in texture were investigated at harvest, after storage and the effect of the year and the rootstock were evaluated. Significant genotypic variation is present in the genepool for texture. The identification of a storage index specifically designed for cherry, based on the texture parameters developed, allows clustering at maturity and after storage the most suitable genotypes for storage attitude or postharvest use.

The texture variability ascertained and association with other quality-related traits in the three berries and in cherry with this research shows that it is underexploited for its use in breeding, thus giving room for future improvements, increasing the chance not only to accelerate progress in selection processes for these novel traits through an appropriate identification and use of them as biomarkers but also allowing a much more focused and assisted process throughout the all industry chain, as for sorting and product segmentation.

Chapter I

Introduction

A market and consumers' perspective of texture in berries

New market exploitation, losses reduction associated with the demand to store products are drivers for the fruit industry (Roger Harker et al., 2003). While it is quite straightforward to assess the economic benefits of improved storage, it is far more difficult to assess the benefits of better overall quality. Food quality, and consequently fruit quality, is a complex and often elusive concept, which includes all those characteristics of a food – not only sensory – with which the consumer is satisfied with the product. Texture, taste and flavour are considered to be the most responsible of fruit quality and the most difficult to assess. It has to deal, of course, with consumers' expectations, beliefs, attitudes, perceptions and it is related to price. In fact, both sensory and emotional aspects of fruit freshness influence consumer perceptions of monetary value too (Lund et al., 2006).

Within this picture, U.S. per capita blueberry consumption increased nearly 600% from 1994 to 2014. Nowadays, berries are products present on the shelves for 52 weeks a year, with peaks highly related to season. Fresh berries sales see the European and US peak season from April to July for strawberry, from June to late August for blueberry, while raspberry starts in July until September, mainly when prices are lower. November, December and January are the most profitable months for berries in the northern hemisphere, although they all tend to reach relatively high prices throughout the whole year (Özen et al, 2014). Sweet cherry has a shorter window of production and higher prices: Russians and Eastern Europeans are the major consumers, followed by other European countries and Latin America. A heavy advertising can stimulate fruit purchasing, but relative price is carefully considered (Richards, 1999). However, as much as the health benefits of berries and superfoods are known, the consumption pattern is changing also concerning price. An experiment conducted on Italian consumers (Di Pasquale et al.,

2011) showed that consumption of functional food was directly correlated with knowledge of the health benefits and not directly depending on the family income and 43% of the consumers were either concerned about their health or fully conscious. Huge differences exist among EU countries (Özen et al, 2014) and age.

The actual fruit industry is experiencing an intense competition, based on price and quality positioning, which push the players to create new know-how, new products, new markets and new alliances through dynamic aggressive dynamics that influence also the research sector, breeding and IP.

Market segmentation and willingness to pay are drivers to reach these new developments. From a breeding standpoint, diversification of taste and texture can be the key to satisfy different consumers' expectations as some prefer firm fruit, other crispy or popping, other melting, other rate more sweetness and flavour intensity.

Taste, freshness, color, and shape/size are positively correlates with purchasing sweet cherry of Japanese consumers (Miller, Casavant, and Buteau, 1986). Californian consumers' choice was positively influenced by SSC, titratable acidity (TA), SSC/TA ratio, and dark skin color (Crisosto et al., 2003), while Michigan consumers decide based on sweetness, flavor, and firmness (Guiyer et al., 1993). Consumers in Oregon are willing to pay a price premium of $0.87 for an extra unit of sweetness and $0.35 for an extra unit of firmness (Hu et al., 2007).

Also in strawberries, freshness, firmness, taste, fruit color, and fruit size are the most important factors for consumer decisions to purchase strawberries (Safley et al., 1999, Lado et al., 2010; Colquhoun et al., 2012) and less willing to buy strawberries with low SSC (Keutgen and Pawelzik, 2007). In summary, the existing literature for strawberry and cherry identifies the importance of quality traits such as texture, flavor and appearance with respect to consumers' intent and willingness to pay price premiums. Wang et al. (2016) identified three categories of strawberry consumers “Balanced Consumers,” “Experience Attribute Sensitive Consumers,” and “Search Attribute Sensitive Consumers.” that can be identified as target markets, but also help breeding to address them.

Significant positive correlations to overall liking of blueberry fruit were found with sweetness, texture, and flavor were found also for blueberry (Gilbert et al., 2015), where panellists could also recognize genotypic differences in blueberry sensory components.

A recent report (Gallardo et al., 2018) analyzed industry responses and highlighted that the most important trait cluster was fruit quality including the firmness, flavor, and shelf life: they affect price premiums received by growers; influence consumer’s preferences and have the potential to increase mechanical harvesting, all critical points into the economic viability of the blueberry industry.

Fruit quality is also tightly connected with its stability during the entire pipeline: traits that facilitate fruit handling like firmness, and for traits linked to grades and standards. The more positive they are, the more they would benefit processing operations. A recent study indicated that also producers preferred to grow firm strawberries with intense flavor, ideal external and internal red color, and longer shelf life (Choi et al. 2017).

When reported to units, a study showed that intermediaries were willing to pay $0.13/lb more to improve fresh apple firmness, $0.009/lb more to improve flavor and $0.002/lb to improve crispness (Gallardo et al., 2015). Sweet cherry intermediaries were willing to pay $0.34/lb to 0,87/lb Hu (2007) for an increase in SSC, $0.31/lb for an improvement in flavor and $0.18/lb for an increase in size. Hu (2007) reported a WTP of $0.35/lb for an extra unit of firmness. Market intermediaries of strawberry were willing to pay $0.24/lb for improved flavor, $0.15/lb for an improvement in firmness, and $0.10/lb for an increase in size (Yue et al., 2012; Gallardo et al., 2012). It is though clear that together with the intrinsic fruit quality, also WTP, consumer likeness, market intermediaries demands need to be taken into account in breeding priorities setting.

What is texture and how it is measured

Texture is one of the most important factors in determining food choice: it is a cognitive property that we subjectively assign to food and it is linked with how our senses interact with the matrix by vision, touch and oral processes. We thus have a feeling of texture before consuming the food, through visual, handling and touching feelings and then during eating.

Texture can be looked at as a subjective complex process which is mostly hedonic, so it has to do with likeness by the consumers. It can also be interpreted as objective when it analytically characterizes the food matrix to be described.

Analytical texture can be sensory, instrumental and a relation of the two. Sensory texture includes mechanical, geometrical and compositional parameters (Waldron et al., 2003). Mechanical texture includes a wide range of parameters that are related to hardness, cohesiveness, elasticity, adhesiveness, viscosity, chewiness and gumminess. Popping and crispiness of a food are in addition related to the acoustic emission of the analysed matrix and involve the auditory perception, either by compression or measured through a microphone, but indexes that use mechanical parameters are also common (Hioshioka et al., 2009). Geometrical texture depends on the size and shape of the food matrix, while compositional texture is strictly and complexly related with the content by means of biochemistry and molecules that lye at the base of the whole process. Instrumental texture can be empirical, based on an imitative test or a fundamental test. Information from empirical texture is mainly subjective but definitely important for example in breeding programs, in which the evaluation and then selection of a high number of seedlings can be the first coming across variability of the complexity of the trait and Elite Line selection. Imitative tests are important because they consider the food matrix in relation to the geometry of the matrix, the ability to fracture and the friction of the sample. By the application of fundamental tests, finally, the opportunity is to clearly identify the units that are fundamental for the process (Waldron et al., 2003). Sensory and instrumental texture can finally together identify structural, mechanical and mouthfeel interactions to advance in quality control, consumer response interpretation and optimized methodology, tailored for the different matrixes.

Taste and flavour, both referring to texture, aroma combinations of blend, association of specific compounds to sugars and acids or volatiles, are difficult to phenotype. The traits are highly influenced by genetics, environment and agronomic practices and individual varying taste. However, modern breeding strictly need to accurate measure large number of plants and plenty of parameters in the most precise way. Technology availability evolves rapidly, giving the opportunity to fine tune, on specific species, protocols that were meant for completely different matrixes. Phenotyping is the set of methodologies and protocols used to measure at different organization scales, from plant organs to plant canopies (Fiorani et al., 2018). The phenotyping bottleneck present in berries requires information on trait development during ripening, at maturation and in post-harvest, because no sound and robust previous literature exist.

Texture and relationship with berries and cherry morphology and softening

Although berries are commercially a single category, they include fruits that are different for their botanical and taxonomical classification. Cherry has commercial affinity to berries, being however a defined and single market category. The Rosaceae family includes strawberry, raspberry and cherry, while blueberry belongs to the Vacciniaceae.

Blueberries, although berry-like, are false berries, that develop from an inferior ovary: Their endocarp is composed of five carpels with ten locules and five lignified placentae to which a variable number of seeds are attached. The fleshy portion of the fruit is represented by the endocarp, surrounded by the mesocarp, which includes parenchymatic cells, vascular bundles, and some stone cells unevenly distributed. From the internal to the external pericarp layers, the endocarp is surrounded by a ring of vascular bundles and the hypodermal layer which is the pigmented part. The epidermis is composed by a single layer of cells without stomata, covered by a cuticle and an epicuticular wax, which contributes to the light blue final color of the fruit.

Fruit development in blueberry follows a double-sigmoid growth pattern in which the pericarp initially increases in volume due to a rapid cell division that occurs after syngamy. In the second lag stage, the embryo and endosperm develop maturing while the pericarp growth is delayed. Ripening occurs when cell expansion of the pericarp occurs, until fruit final size is reached and pigmentation completed (Godoy et al., 2008; Blaker et al., 2014).

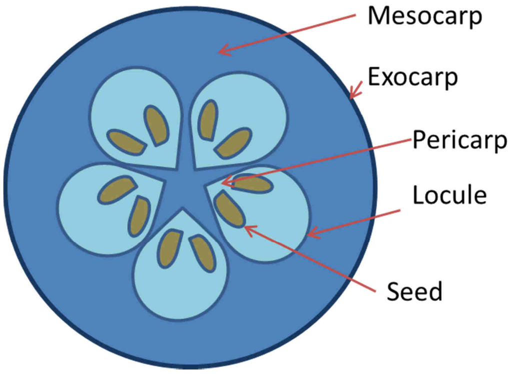

Figure 1.1 Fruiting *corymbus* of a northern blueberry.

Figure 1.2 Schematic graphical section of a blueberry fruit. Tissues can vary among genotypes and species.

During physiological ripening in blueberry, the total water soluble pectin decreases and the degradation of the cell wall and middle lamella is responsible for the loss of firmness. Texture in blueberry depends on several anatomical features, such as the tissue layers and cell size (Jackman, 1995; Chiabrando et al., 2009; Klima Johnson et al., 2011), and is the result of a combination of different sub-phenotypes, such as firmness, mealiness, gumminess and juiciness.

Raspberry (*Rubus idaeus* L.) is an aggregate of drupelets (Fig. 3), held together by interlocking hairs forming an aggregate fruit, adhered to a variably conic receptacle, from which it separates when ripe. Raspberry is characterized by a rapid ripening rate and a very limited storage attitude. Softening due to excessive loss of firmness strongly limits its storage, transport, and marketability. As resumed by Vicente (2007), fruit firmness may be influenced by altered hydrostatic turgor within fruit cells (Shackel et al., 1991). Membrane damage and mesocarp cell modifications could be involved in textural changes (Sexton et al., 2007). However, changes in the composition and architecture of the cell wall are the most responsible of the decay. Pectic compounds undergo during ripening the most extensive modifications among the cell wall polymers: the solubility of cell wall polyuronides increase and is accompanied by a dramatic depolymerization, while the metabolism of hemicellulosic polymers is not a cardinal factor (Vicente, 2007).

Red raspberry fruit have been classified as climacteric (Blanpied, 1972; Jennings, 1988) and nonclimacteric (Kader, 1985) relative to color development. However, in a study on Heritage by Perkins-Veazie et al. (1992), over six stages of fruit development data showed that ripening in raspberry fruit is independent of C2H4 production and is nonclimacteric. Soluble solids concentration and fruit weight increase, titratable acidity decreases during ripening.

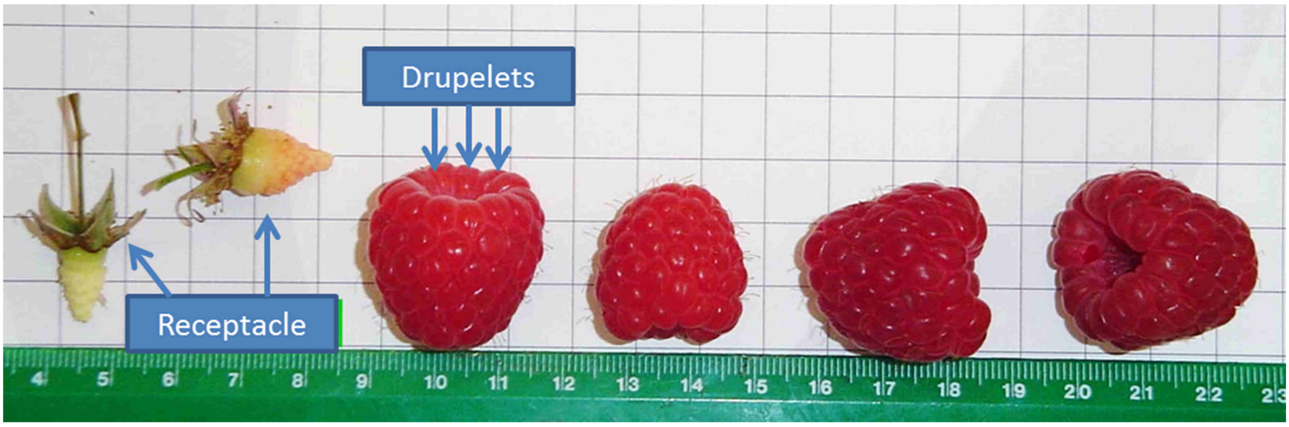

Figure 1.3 Raspberry ripe fruit and inner receptacle.

Strawberry (*Fragaria* x ananassa) fruit initiate from a single inflorescence and are actually an aggregate, composed of many ovaries, each with a single ovule (Perkins-Veazie, 1995). The true fruit of strawberry (Figure 1.4) are the seeds, called achenes, which are embedded in the epidermis of the swollen receptacle. The receptacle is composed of an internal pith, a cortex layer, and an epidermal layer (Suutarinen et al., 1998; Fait et al., 2008). Fibrovascular strands connect the achenes to the interior of the fruit, supplying nutrients to the achenes and the parenchyma cells. The achenes vary for position, size, shape and color, according to the cultivars. The mature achenes contain a relatively thick pericarp, a single layer endosperm, and a small embryo, whose formation is completed 10 days after anthesis (Nitsch, 1950). The false fruit, represented by the fleshy receptacle, follows mainly a single sigmoid curve pattern of development and growth. However, double sigmoid patterns have been reported for some cultivars.

In the first lag phase, auxin, synthesized in the achenes, promotes fruit growth, reaching a peak in concentration prior to the white stage, later declining as the fruit matures. The subsequent changes during strawberry maturation are accompanied by coordinated modifications in the levels of transcripts related to primary and secondary metabolism (Aharoni and O’Connell, 2002). During the first developmental stage, the length of cell division plays a relevant role in determining the final fruit density at harvest, and several fruit quality traits are strongly linked to this parameter (Farinati et al., 2017). Phytohormones greatly impact the faith of this development too: although the Rosaceae family, which includes strawberry, cherry and raspberry offers a multitude of fruit development patterns, common set of molecular and physiological events to achieve the final product can be found, not only for the sigmoid or double sigmoid patter, but also according to the hormonal content trends that can be drawn for the different fruit types (Rasori et al., 2010; Eccher et al., 2008; Farinati et al., 2017).

Figure 1. 4 Fruit of the strawberry cultivar Elsanta.

The major soluble sugars in strawberry are glucose, fructose and sucrose, which significantly increase during the development (Hancock, 1999), while minor soluble sugars, like inositol, xylol and galactose decrease (Moing et al., 2001). The main organic acids of strawberry are citrate, malate, and quinate, while minor organic acids include acetate, oxalate, succinate, isocitrate, fumarate, and aconitate (Moing et al., 2001). Sugar and ratio between sugars and acids are major components of fruit quality determination and consumer acceptance (Park et al., 2006). Amino acids are other soluble gear to fruit flavor, together with phenolic compounds. Phenolic compounds contribute to fruit color, flavour, and interact with mechanisms of pathogenic and UV protection (Aaby et al., 2005). They also form on a two phase pattern (Halbwirth et al., 2006) with flavanols, mainly tannins, accumulating during early stages to high levels and providing to immature fruit an astringent flavor (Aharoni et al., 2002; Almeida et al., 2007). In later stages, other flavonoids, such as anthocyanins and flavonols show a second peak (Almeida et al., 2007; Landmann et al., 2007). A model for the cross talk between primary and secondary metabolism was proposed by Fait et al. (2011) with the aim of addressing the metabolic regulation underlying fruit seed development and the results suggest that changes in primary and secondary metabolism reflect organ and developmental specificities, highly coordinated during early development.

Nonetheless, a comprehensive view of the metabolic changes in the strawberry network during fruit development is still not completely defined and in particular for texture.

Mechanisms underlying the hormonal, transcriptomic and proteomic modifications occurring during fruit development, epigenetic mechanisms underlying heritable traits of agronomic importance are studied, with particular regard to the exploitation of economically important phenotypes through breeding (Farinati et al., 2017). Both during development and post-harvest, how gene regulation is driven by molecular mechanisms remain a strong topic to be studied. Mechanisms leading to the alteration of the structure of chromatin and changes in the subsequent gene expression, that can include DNA methylation, Post-Tranlational Modifications, which are highly organ specific, also in other crops (Bonghi et al., 2011), need to be further understood in their complexity but undeniable contribution to the fruit quality determination.

Cherry is a drupe (Figure 1.5), mostly flat round in shape, with variable colour from cream to vermilion of the flesh and skin of the internal and external tissues. The drupe is adhered to a stalk that is variable in length. Size varies according to the cultivar as well as chemical values. Total acidity shows differences occurred among sweet cherries, as well as sugars levels. The flesh is usually soft, although "popping" varieties are known.

Epidermal characteristics are related to cracking: Demirsoy et al. (2005) found a negative correlation between the cuticle thickness and fruit cracking and no correlations of fruit cracking with thickness of epidermis, subepidermis and number of subepidermal cell layers.

Peschel et al. (2007) dissected the wax and cuticle composition, and at maturity, found that triterpenes, alkanes and alcohols accounted for 75.6%, 19.1% and 1.2% of total wax. The cutin fraction of mature fruit consisted of mostly C16, C18 monomers including alkanoic, ω-hydroxyacids, α,ω-dicarboxylic and midchain hydroxylated acids.

Figure 1. 5 Sweet cherry fruit (cultivar Kordia)

The two principal morphological components of sweet cherry are the seed and the pericarp. The seed embeds the endosperm, the embryo and the seed coat. Externally, the pericarp is composed by the endocarp, the mesocarp and the exocarp.

According to Tukey (1939), the stony pericarp may be divided into an inner and an outer layer, as well as the fleshy pericarp. The inner layer of the fleshy pericarp is made of small thin-walled parenchyma, while the outer layer is made of collenchyma. Fleshy and stony pericarp derive from distinct groups of cells. At maturity cells show an increase of twenty-five times in diameter from the size at full bloom: they also increase in number, the epidermal ones in particular, increase in size and wall thickness Tukey (1939).

State of art of texture berries breeding and genetic knowledge

Berries breeding is facing today a new paradigm. The major customer of the breeder and the grower is becoming the consumer, which is a complex client according to taste, habits but also traditions and culture. If until now, yield, cost of labour, resistance to pests and postharvest handling were a priority, now a consumer assisted breeding is more common. Often, the supermarkets act as interlocutors to breeders as well as to growers through them, asking for what are the most priceable traits that their end consumers ask for. Flavour is certainly the most stressed trait, in particular for berries. Growers are not paid for flavour quality, thus they do not demand it, but surely supermarkets are. If until yesterday the consumer was not included in cultivars development, now this category, in its multifaceted aspects, is the focus for many breeding programs. Growers cannot be left out of this development, but they mark less than before their influence on higher quality products.

Genome sequencing and GWAS approaches have made huge improvements in accessing more information and reduced the cost toward an approach that includes now possibility to define the biochemistry of consumer preferences and liking, identifying those genes and the alleles of them that contribute to favourable new products.

Flavour is the sum of inputs informing our brain of what we are eating through multiple senses (Klee et al., 2018). Taste, through the five classes of mouth receptors, refers to levels of sweet, sour, salty, bitter and umami. Smell is fundamental for flavour perception recognizing the volatile compounds through the olfactory receptors in the nasal epithelium.

In human, more than 400 olfactory receptor genes are documented, being humans able to distinguish on average 1 trillion of smells. This has necessarily to be taken into account when evaluating the variability of a subjective measure of a food matrix, where this processing ability is augmented, becoming even more complex due to the interaction with the food matrix. In blueberry more than 300 volatile compounds were detected, more than 400 in strawberry and raspberry, with an interplaying activity that is not stable or “syntenic” for the different crops.

Texture and appearance are the other two keys to perception of a food matrix, through touch, acoustic and visual senses, being texture one the most determining (Pascua et al., 2013).

Different compounds and mechanical parameters of the food eaten, act combinatorially to trigger response also at subthreshold levels. Also, some volatile compounds over a threshold may have a negative impact on the flavour perception.

The structure and chemistry of each fruit is thus unique, imparting characteristics that are associated with the species and the cultivar and most important are associated to liking.

Different critical steps have thus to be taken into account analysing berries. Blueberry, strawberry and raspberry can have the advantage to produce multiple production cycles over the year.

However, berries breeding deals with a previous genetic knowledge that is different for the different species.

Genetic improvement of fruit softening has been largely studied in different fruit models and crops. Softening in fruit depends on the disassembly of polysaccharide-rich cell walls, diminishing of cell-to-cell adhesion and changes in the most external tissues, namely cuticle and skin, that affect water loss (Seymour et al., 2013; Martin et al., 2014).

A plethora of literature exist that studies the mechanism involved in this process (Uluisik et al, 2016), but precise answers still remain elusive.

Genes involved have been found. In tomato, the use of *ripening inhibitor (rin)* mutation has been widely used in breeding to confer long shelf life, affecting texture. Now, studies on pectate lyase (PL) seems also to be a crucial major gene for fruit softening, whose silencing does not affect other ripening aspects, like texture (Uluisik et al., 2016). The tomato genome sequencing allowed to reveal more than 50 structural genes encoding proteins involved in cell-wall modifications during fruit ripening. The most expressed were polygalacturonase (PG), pectin methyl esterase, B-galactanase and expansin and all have been studied as candidates in texture fruit changes. However, silencing their expression has not yet lead to detectable modifications of softening (Smith et al., 2002; Tieman et al., 1994).

On the contrary, PL silencing in strawberry reduced fruit softening (Jimenez-Bermudez et al., 2002).

In transgenic tomato lines*, PROTODERMAL FACTOR 2-like* and *CER1* were shown to be involved and upregulated in tomato: the two genes encode proteins involved in regulating epidermal and cuticle development, which might have an effect on water loss and fruit shelf life. Of course, the anatomical structure and tissues of tomato are different than strawberry, thus further studies are certainly needed.

Blueberry, for example, is an autotetraploid species that is still lacking sequencing information on a large scale. The commercial blueberries are now mainly two types or hybrids of them, whose genetics studies are still far to be directly diagnostic. MAB has produced valuable results in terms of chilling requirements, cold tolerance, late fruiting and tart fruit (Rowland et al., 2014). A genetic linkage map of a highly variable F1 has provided with QTLs that are involved in firmness, fruit quality, plant size, yield, buds and rate of flower.

From the phenotyping results of a northern x southern highbush cross, data appropriate for a QTL analysis was published (Hancock et al., 2018). Here, fruit firmness, fruit scar and % of juice were not significant in the interaction between genotype and environment, while genotypic differences among individuals were significant. Another SNP and SSR linkage map was published using genotyping by sequencing (McCallum et al., 2016).

Other sequencing efforts are made, whose results will be available soon: one mainly private, thus of more difficult use by different breeding programs over the world due to proprietary IP law, and other public, which see the involvement of different multidisciplinary groups to afford the high costs of the process and combine the capacities of approach to a polyploid species.

Rosaceae firmness genetics, thus including strawberry and raspberry is only a little clearer. Strawberry, cherry and raspberry are all Rosaceae species, which includes some 90 genera containing approximately 3000 species (Longhi et al., 2014). Some of them are of economic high importance such as cultivated apple (*Malus pumila* Mill.), pear (*Pyrus* spp.), peach (*Prunus persica*), sweet cherries (*P. avium*) and roses (Rosa spp.). The base chromosome number is x=7. The genomes estimate for *Rubus* species ranged from 0.58 pg/2C for R. ideaus to 0.75 pg/2C for R. sanctus. The genome size estimate of *F. vesca* was determined by flow cytometry through comparison with the 125 Mbp/C sequenced genome size of Arabidopsis thaliana to be in the region of 206 Mbp/C (Longhi et al., 2014). The genome size of the cultivated octoploid strawberry was also estimated in the region of 703 Mbp, less than four times the estimated genome size of the diploid Fragaria, suggesting extensive loss of chromosomal content following polyploidisation. Thus, Rosoideae genomes have been estimated to be among some of the smallest of all angiosperm genomes, with Fragaria having the smallest, followed by Rubus (Dickson et al., 1992; Meng et al., 2002; Akiyama et al., 2001).

Zorrilla-Fontanesi (2011) studied the genetic control of 17 agronomical and fruit quality traits: together with plants characteristics and yield, fruit firmness using a penetrometer, soluble solids content and fruit color were measured for QTL analysis and 33 significant associations with QTLs were found. A QTL for fruit firmness was confirmed, that was previously located in the same chromosomic region as Fa-Exp2, an expansin specifically expressed in strawberry fruits (Dotto et al. 2006). The presence of homoeo-QTLs, that is, QTL controlling a particular trait that mapped to orthologous positions on homoeologous linkage groups, was also confirmed. In the two different progenies studied Zorrilla-Fontanesi (2011), plant width, fruit shape, firmness, glucose and malate content, pH, terpenes linalool and terpineol were shown to be controlled by homoeo-QTLs, suggesting that more than one homoeologous gene copy regulates the expression of particular quality traits, and raising implications for marker development for MAS.

To date, no data on the genetic determinism in on sweet cherry is available for both fruit firmness and size (Campoy et al., 2015) . In peach, QTLs for firmness were identified (Cao et al., 2012a), and candidate genes (CG) for texture were mapped on all eight LGs (Illa et al., 2011; Ogundiwin et al., 2009). The locus responsible for the melting vs. non-melting (M/m) flesh character has a major effect on fruit texture and firmness in peach and has been mapped on LG4 (Cantin et al., 2010; Dirlewanger et al., 2004; Peace et al., 2005). Synteny of this region was found with apple, where a cluster of QTLs for fruit quality traits, including firmness (Kenis et al., 2008). In apple, a polygalacturonase gene (Md-PG1), known to be involved in cell wall metabolism processes, is also mapped on this interval (Longhi et al., 2012).

Aims of the study

Cherry and berries, including strawberry, raspberry and blueberry are increasingly demanded fruit on the market. They are a ready to eat category, whose storage attitude is quite short, being highly perishable.

Fruit quality and consumer liking suggest that genetic improvement of texture and fruit quality texture-related traits is challenging.

The aim of this study was to implement the traits primarily involved in the “quality concept” profile of each crop through instrumental achievements and objective assessments in order to focus their breeding.

A classification, derived from a precise texture clustering ability of the different berries within different genera (eg: *Fragaria, Rubus, Vaccinium, Prunus*) would allow a better definition of groups of fruits with similar characteristics related to the architecture of the cellular layers composing the fruit and or secondary metabolites presence. This would allow to select fruits with different texture and quality characteristics suitable to different markets (eg: gummy fruits or juicy vs crispy ones or different flavours or anthocyanins content). On the other side, the ability to develop precise classifications would bring advantages to the whole production pipeline.

In particular, my PhD work aimed to answer four main research questions.

1. **Is there an analytical genotypic variability of texture and other quality traits texture-related in the berries germplasm of blueberry, raspberry, strawberry and cherry?**
2. **Is this genotypic variability stable from harvest to post-harvest?**
3. **Are pre-harvest and post-harvest factors influencing texture and the other traits?**
4. **Is it possible to implement through the use of this information the breeding programs of these crops?**

I attempted to dissect texture and only partially its relationship with other quality traits, in spatially distinct parameters in different berries species with different fruit anatomies.

The primary milestone was to define protocol optimization for group species, setting the infrastructure pipeline in a view of routine phenotyping workflow. From the measurement of phenotypic parameters, through assays and protocols, data were then evaluated in order to profile specie-specific response models, to be finally used for G or GXE analyses.

Some keywords are thus recurrent in this study, namely texture, fruit quality, berries and breeding; they have been encompassed the introduction, and furtherly discussed in a more species-tailored way in each of the following chapters.

Chapter II

Fruit quality profiling of texture in blueberries for parental choice in breeding

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GL conceived and designed the research, coordinated the experiment, analyzed the data and wrote the manuscript. Most of the chapter refers to the he original publication, available at Giongo, L., Grisenti, M., Khomenko, I., Poncetta, P., Loretti, P., Ajelli, M., Biasioli, F. and Farneti, B. (2017). Fruit quality profiling of blueberries for parental choice in breeding: aroma and texture at harvest and in postharvest. Acta Hortic. 1180, 197-204 DOI: 10.17660/ActaHortic.2017.1180.26 https://doi.org/10.17660/ActaHortic.2017.1180.26, for which permission was granted by the editor, while a part is an implementation more recently achieved. All the authors contributed to the research. AM ran the texture samples. GM sampled most of the samples. PP sampled fruit and propagated the plants. SP revised the manuscript. FB contributed to the statistical analysis, analysed and interpreted the volatile data and contributed to writing of the original manuscript.

INTRODUCTION

Enhanced knowledge about blueberry germplasm diversity and genetic relationships among plant breeding materials could be an important support to crop improvement strategies. The simplest way of selecting superior parental lines is to choose them, based on their performance related to the traits of interest. The higher the trait is heritable, the more the selection can be reliable and efficient (Valdomiro et al., 2000), while it becomes unproductive with low heritability (Falconer, 1989). Phenotypic diversity are nowadays possible to be assessed in different ways, that can rely on pedigree information, morphological and agronomic features, biochemical data and, more recently, a combination, on different extents, of more than one of them together. Genetic diversity can be combined and associated to the phenotype, by the use of several routine molecular techniques, first of all by fingerprinting through SNP and/or SSR.

Choice of genetic distance measures, objective determination of genetic relationships, and multivariate methods of data analysis, such as hierarchical clustering, are key elements for blueberry improvement. The importance of these methodological approaches is related to the possibility to gather many variables into one analysis, allowing the breeder to choose the best parents for controlled crosses. Parental choice, as well as Elite Line selection, is thus a key step in breeding, with particular regard to traits that are still poorly defined and studied in blueberries (*Vaccinium* spp.) like texture, which play a crucial role in the determination of the consumer acceptance. However, breeding for these quality traits, commonly assessed subjectively, is not an easy task: the biochemical components in blueberry that contribute to flavour, texture, thus taste are directly regulated by the effects of genotype genetic differences (G), by the production environment (E), and also by the G×E interactions. In breeding fruit for quality traits a consistent performance of a cultivar between growing locations and seasons is desirable. Therefore, in this study we tried to identify those parameters for texture whose expression is mainly regulated by genotype than environment or G×E interaction effects. Breeding selection of these traits are generally based on non-analytical observations assessed at a unique data point - mostly recorded at harvest - which makes extremely difficult to precisely address specific parameters to be ameliorated. The aim of this work was to obtain an high throughput quality profiling of blueberries, with regard to complex and commercially important traits like texture and its contribution to taste and flavour, in order to unravel the variability present in the germplasm and use the information for breeding advancements, at ripening stage and during postharvest.

MATERIALS AND METHODS

Plant material and sampling

46 *Vaccinium* accessions (Table 1) were chosen from the experimental field of FEM Research and Innovation Centre at Pergine (Trento), located in the north of Italy (Trentino Alto Adige region- 46.0744° N, 11,2334° E, 525 m a.s.l.). At the time of the analysis, plants were in the full production phase, between 7 and 10 years old. Fruits were harvested at maturity stage assessed according to the method described by Giongo et al. (2013), coincident with commercial harvest. Homogeneous fruits were sampled immediately at harvest and divided into two batches, for the harvest timepoint analysis and postharvest ones, of about 80 fruits each. Analyses were carried out at harvest and after six weeks of storage, at 2°C.

Additional 12 putative varieties were collected from various supermarkets in the UK. According to their labelling, they were Aroma, Blue Aroma, Corona, Divina Blue, First Blush, Juanita, K42, Magna, RC1, Rocio, Romero, Snowchaser, Star, Stella Blue, Sundown, Suzie Blue, Ventura. Time from harvest was for all between 1 and 2 weeks and provenience was different. Analyses were conducted on 30 fruits each variety, separately from those grown in Italy. A preliminary comparison on the main traits (texture and size) as indicative general mean was conducted, considering them the newest commercial releases of blueberry actually present on the EU market.

The advanced selections of the breeding programs at FEM were also analysed. They were all grown in 30 liters pots, conducted in soilless conditions.

Pedigree preliminary analysis

Vaccinium accessions selected for this study were chosen based on the pedigree analysis assessed by PediMap (Voorrips, 2007). Acronyms of six to nine letters were adopted for lengthy cultivar names. Published pedigrees used for verification were sourced from the following: GRIN (Germplasm Resources Information Network; http://www.ars-grin.gov) and from the Brooks and Olmo Register of Fruit and Nut Varieties (Lists 34-47) and specific patents.

Table 1 List of the blueberry cultivars evaluated at harvest and during post-harvest through texture analyses.

Number of corresponding bars order	**Cv**	**Ancestors**	**Number of corresponding bars order**	**Cv**	**Ancestors**
1	Aron	*V. angustifolium V. uliginosum*	**25**	Jubilee	*V. corymbosum V. darrowii V. elliotti*
2	Atlantic	*V. corymbosum*	**26**	Legacy	*V. corymbosum, V. darrowii*
3	Aurora	*V. corymbosum*	**27**	Liberty	*V. corymbosum*
4	Azur	*V. corymbosum*	**28**	Marimba	*V. corymbosum V. darrowii V. ashei*
5	Berkeley	*V. corymbosum*	**29**	Misty	*V. corymbosum, V. darrowii*
6	Biloxi	*V. corymbosum, V. virgatum, V. darrowii*	**30**	Northblue	*V. corymbosum; V. angustifolium*
7	Bluecrop	*V. corymbosum*	**31**	Northland	*V. corymbosum; V. angustifolium*
8	Bluemoon	*V. corymbosum*	**32**	Nui	*V. corymbosum*
9	Brigitta Blue	*V. corymbosum*	**33**	O'Neal	*V. corymbosum V. darrowii V. ashei*
10	Centrablue	*V. virgatum*	**34**	Ozarkblue	*V. corymbosum*
11	Centurion	*V. virgatum*	**35**	Poppins	*V. corymbosum V. ashei*
12	Chandler	*V. corymbosum*	**36**	Primadonna	*V. corymbosum hybrid*
13	Compact	*V. corymbosum*	**37**	Puru	*V. corymbosum*

14	Cosmopolitan		**38**	Roxyblue	*V. corymbosum V. darrowii V. elliotti*
15	Coville	*V. corymbosum*	**39**	Rubel	*V. corymbosum*
16	Darrow	*V. corymbosum*	**40**	Safir	*V. corymbosum*
17	Earlyblue	*V. corymbosum*	**41**	Simultan	*V. corymbosum*
18	Elizabeth	*V. corymbosum*	**42**	Skyblue	*V. virgatum*
19	Elliott	*V. corymbosum*	**43**	Southernbelle	*V. corymbosum V. darrowii*
20	Emerald	*V. corymbosum V. darrowii V. elliotti*	**44**	Star	*V. corymbosum V. darrowii V. ashei*
21	Mondo	-	**45**	Top Hat	*V. angustifolium*
22	Goldtraube	*V. corymbosum V. lamarkii*	**46**	Toro	*V. corymbosum*
23	Jersey	*V. corymbosum*			
24	Jewel	*V. corymbosum V. darrowii V. elliotti*			

Texture analysis

Texture assessment was performed on 20 homogenous fruit at harvest and after 6 weeks of cold storage (RH 85% and 2 °C). Texture was profiled by a texture analyzer (Zwick Roell, Italy) and data were analysed by TaxtExpertII software.

The penetration texture analysis outlined a mechanical force displacement using a 5 kg loading cell and a cylindrical flat head probe with a diameter of 4 mm entering into the berry flesh from the sagittal side. The mechanical profile graph was based on two fundamental variables: force (N) and distance (strain, %). Mechanical profiles were acquired with a resolution of 500 points per second with the following instrumental settings: test speed of 100 mm/min, post-test speed of 300 mm/min, auto force trigger of 2 g and stop plot at target position. Each berry was penetrated until a 90% penetration strain.

The penetration test was applied on the 46 cultivars a with a setting of the parameters according to Giongo et al., 2013. They were designed based on the force displacement profile: maximum force (F_Max, N), minimum force (F_min, N), final force (F_fin, N), area (Area, Nmm), gradient parameters indicating the Young's modulus (G_YM, MPa), deformation at maximum and minimum force (Def_Fmax and Def_Fmin both expressed in %).

The 46 cultivars were evaluated also after 6 weeks of cold storage, to simulate shipping conditions. Based on these results the storage index (SI) was computed using the formula (Giongo et al., 2013), SI = log2(TiPH/TiH), where TiH is the value of the 'i' texture parameter measured at harvest, and TiPH is the value of the same parameter measured after cold storage. Positive SI values indicate a texture sub-trait enhancement, whilst negative values point to a loss of textural performance during storage. An SI equal to zero means stable maintenance of the texture sub-traits under investigation.

Statistical analysis

Multivariate statistical analysis have been performed employing R package ''ChemiometricsWithR'' on Log transformed data. Data were also analysed through Statistica 13.

RESULTS AND DISCUSSION

Germplasm for parental choice

The blueberry germplasm analysed for this experiment consisted on old and new commercial cultivars. A subset of cultivars, indicated as blue flags in Fig. 1, was chosen independently from texture or firmness characteristics, but to be representative of heritability and of the two main groups of blueberries: Northern Highbush Blueberry and Southern Highbush Blueberry together with a number of known hybrids between the two. This classification is mainly based on the chilling requirements, but it represents also a differential introgression of species. NHB is adapted to temperate climates, of about 600-1200 chilling hours, while SHB, developed by the introgression of *Vaccinium darrowii* Camp. and *Vaccinium elliottii*, native of the southern part of the US, is more adapted to warmer climates. Fruit quality of these fruits is diverse (Ehlenfeldt et al., 1995; Hancock et al., 2008). The PediMap result allows to graphically view the coverage of the analysis on the complete germplasm and present the genetic information in pedigrees (Fig. 2.1).

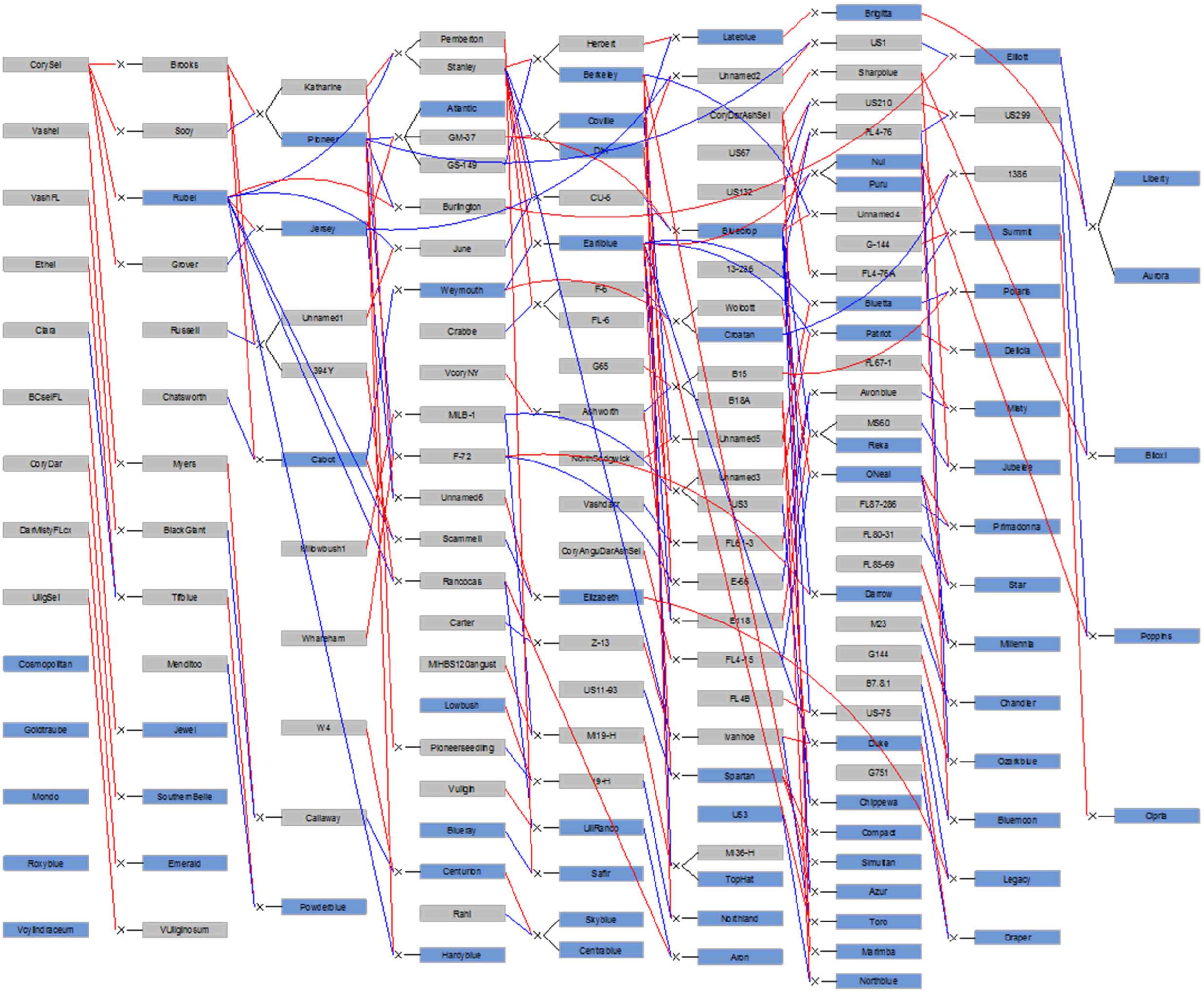


Figure 2.1 Coverage of the analysis on the blueberry germplasm and presentation of the genetic information in pedigrees. Blue flags represent the cultivars chosen to be assessed for texture analysis.

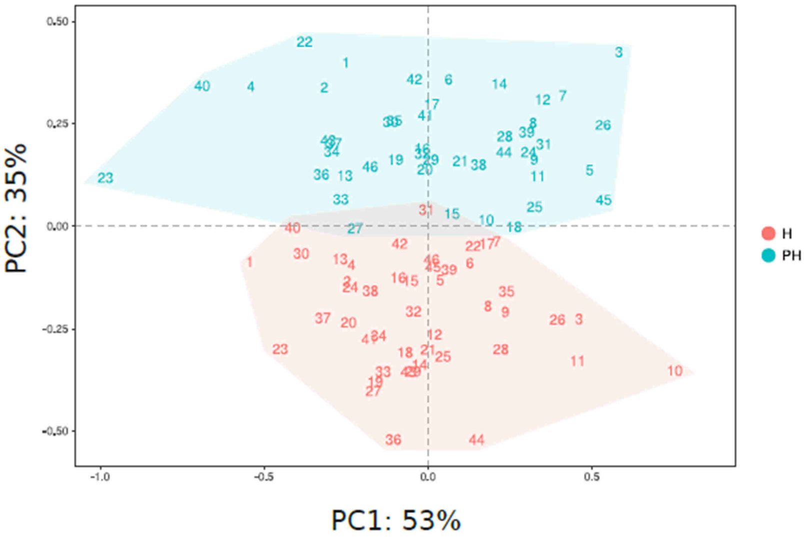
Texture comparison at harvest and post-harvest

To profile the blueberry texture in a consistent scenario of germplasm, the set of 46 cultivars chosen after PediMap analysis underwent texture mechanical analysis. The texture variability, assessed at harvest and after 6 weeks of storage, is represented by a PCA plot (Fig. 2.2a) defined by the first two PCs (PC1: 52,6% and PC2: 33,6%). Texture parameter projections are shown in the loading plot (Fig. 2.2b) and their setting and meaning is resumed in Table 2.2.

Mechanical parameters	**General description of the parameters**	**Unit**	**Curve**	**Acronym**
Max force	Maximum force value recorded over the probe's travel	N	Force-deformation	FMax
Min force	Minimum force value recorded over the probe's travel	N		Fmin
Final force	Force measured at the end of the probe's travel	N		Ffin
Area	Area underlying the mechanical profile	N mm		Area
Deformation at maximum force	Computation of the maximum force associated with the curve on its whole length	%		Def_FMax
Deformation at minimum force	Computation of the minimum force associated with the curve on its whole length	%		Def_Fmin
Gradient	Young's modulus or elasticity modulus, computed as ratio between stress and strain	MPa		G_YM

Table 2.2 List of the main mechanical parameters related to the texture blueberry profiling and relative characteristics applied through penetrometer, on the set of cultivars at different stages and in post-harvest.

The multivariate analysis of the texture data explains thus 86,2% of the total variation for texture. The gradient parameter, also called Young's modulus, is almost orthogonally oriented with respect to the other force related parameters, and opposite to the deformations at minimum and maximum forces. Some single texture parameter explains thus a significant part of the total texture variability, with a level of redundancy for the force parameters, namely maximal force, minimal force, area and final force, that group almost together. The specific orientation of these parameters clearly categorized the general texture performance variability at harvest and postharvest into two main groups (red and blue respectively in Fig. 2.2).



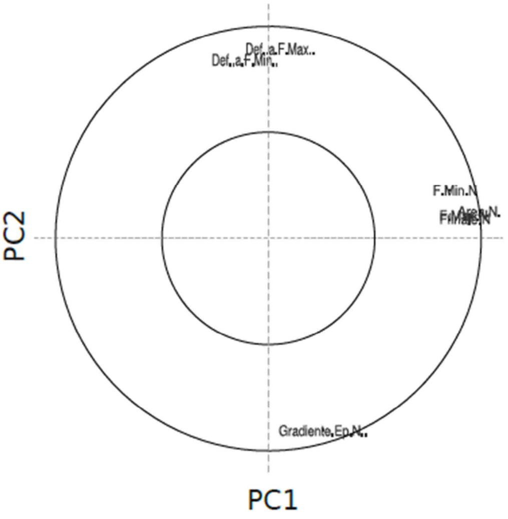


Fig. 2.2a Principal Component Analysis representing the distribution of the blueberry cultivars at harvest (in red) and postharvest conditions (in blue)

Fig. 2.2b Relative loadings of the texture parameters through penetrometric test.

The variability between these two groups is mainly explainable by the PC2 variability, while the PC1 variability remained almost unchanged after the fridge storage. As initially supposed, this broad genetic variability leads to a high phenotypic variance that is enhanced and altered by prolonged cold storage.

The main discriminating parameters are represented by the gradient and, in opposition to it, by the forces deformation. A mechanical profile with a decreasing trend means that the initial parameter, represented by the Young's modulus (or elasticity in this case) is higher than the final. The first group (at harvest) is mostly characterized by more turgid fruits distinguished by a high internal turgor pressure, and a clear variable elasticity within the group. This elasticity, mostly related to the external tissue layers of the fruit, increases during storage.

Textural differences related to cold storage are mostly explainable by the second component (PC2) variability built upon differences in deformation and Young's module, while differences among Vaccinium genotypes are for the most related to the first component (PC1) that is highly correlated to the deformation forces (maximum and minimum forces). In accordance with results presented in Giongo et al. (2013) the Young's module is orthogonally oriented to the force related parameters and nearly oppositely oriented to deformation strains. This observation can be explained by the physical properties of the samples. The perception of a gummy berry is associated with an increased deformation strain at the maximum force caused by a lower turgidity and a high resistance against the force required to break the skin.

Based on the orientation of these seven textural parameters the blueberry collection can be categorized into four main textured types.

The first group, mostly distinguished based on the gradient variability, is characterized by turgid fruit with a high internal turgor pressure, while the second group is mostly composed of firm, rather than turgid, fruit. The last two groups are instead defined by low texture performance berries, for both the Young's modulus and deformation forces, leading to the perception of gumminess. The texture analysis indicates a strong cultivar-storage interaction that suggests the need, in order to fully assess blueberry texture, to strictly consider both the timepoints of analysis.

After 6 weeks of cold storage, the deformation of all cultivars increased, without any significant relation with the values recorded at harvest. Several accessions, such as Star, Elliott, or Chandler, are defined by low deformation levels at harvest and very high values after storage. Differently, other cultivars characterised by low deformation at harvest, such as Centrablue, do not considerably change during storage. Accessions defined by high deformation at harvest also show the same variability, with cultivars stable during storage, like Biloxi and Compact, or very unstable ones like Jersey and Azur. The overall increase in deformation caused by prolonged storage can be mostly explained by a turgidity decrement of the blueberries that lost between 6% to 15% of water during storage. However, fruit weight loss and deformation fold changes are not strongly correlated.

Maximum force variability of fruit assessed at harvest ranges from around 2.7 N (cv Jewell) to 5.1 N (cv Centrablue). Differently from the deformation results, the maximum force variability, assessed after storage, increases. This high variability is mostly explainable by both positive and negative changes during storage. Among the accessions that show low force values at harvest, like Jewell and Jubilee, the first decreases the force after storage, while the second more than doubles its value. Likewise, among those accessions defined by high force level at harvest, Centra Blue decreases, while Brigitta Blue increases. In addition, several accessions, like Biloxi and Centurion, show a very stable force level during storage with minimal modifications.

The Young's module (gradient) values are highly negatively correlated with deformation as previously supported by the PCA analysis. Young's module ranges from around 1.0 MPa (cv Northblue) to 2.2 MPa (cv Centrablue) at harvest. After 6 weeks of cold storage, the Young's module of all cultivars decreased, without any significant relation with the values recorded at harvest. Although several cultivars revealed opposite trends for both Young's module and deformation during storage, such as Ozarkblue, Top Hat, Cosmopolitan, for other cultivars, as Centrablue and Berkeley these values are not comparable. For instance Centrablue is the cultivar with the strongest Young's module decrement during storage and, at the same time, one with the lowest deformation changes. Firmness and softening changes mainly depend on hemicellulosic depolymerization (Vicente et al., 2007), while the gradient (elasticity/turgidity) is more related to the internal turgor pressure regulated by cuticular wax properties (Fava et al., 2006) preventing water loss (Davies and Flore, 1986; Connor et al., 2002), which can be regulated by both genetic and cultural factors (Sams, 1999; Ehlenfeldt and Martin, 2002; Perkins-Veazie et al., 2008; Rodarte Castrejón et al., 2008; Saftner et al., 2008). The relative position of each cultivar is thus representative of peculiar texture characteristics in the two time-points, that have to be considered for cross planning because they indicate distinct and cultivar specific dynamics, critical for the selection of the most favourable individuals.

The dendrogram related to the Storage Index (Fig. 2.3) is intended to describe the potential storability of each accession. The SI provides valuable information related to the magnitude of the variation of each texture parameter during storage, rather than an absolute value.

Three distinct clusters of cultivars are statistically distinguished mostly based on their texture variability. Accessions of the second cluster (red), such as Biloxi, Centrablue, or Ozarkblue, show low delta of the values of maximum force, and intermediate of the deformation at maximum force and of the gradient. The second cluster (blue) is characterized by cultivars, such as Star, Aurora, and_Elliott, with high delta of the deformation of the maximum force values, low delta of the gradient, and an intermediate delta of the forces. Cultivars of the last cluster (green), such as Jubilee, Berkeley, or Brigitta Blue, show high changes of gradient values and forces, and low changes of the deformation at maximum force.

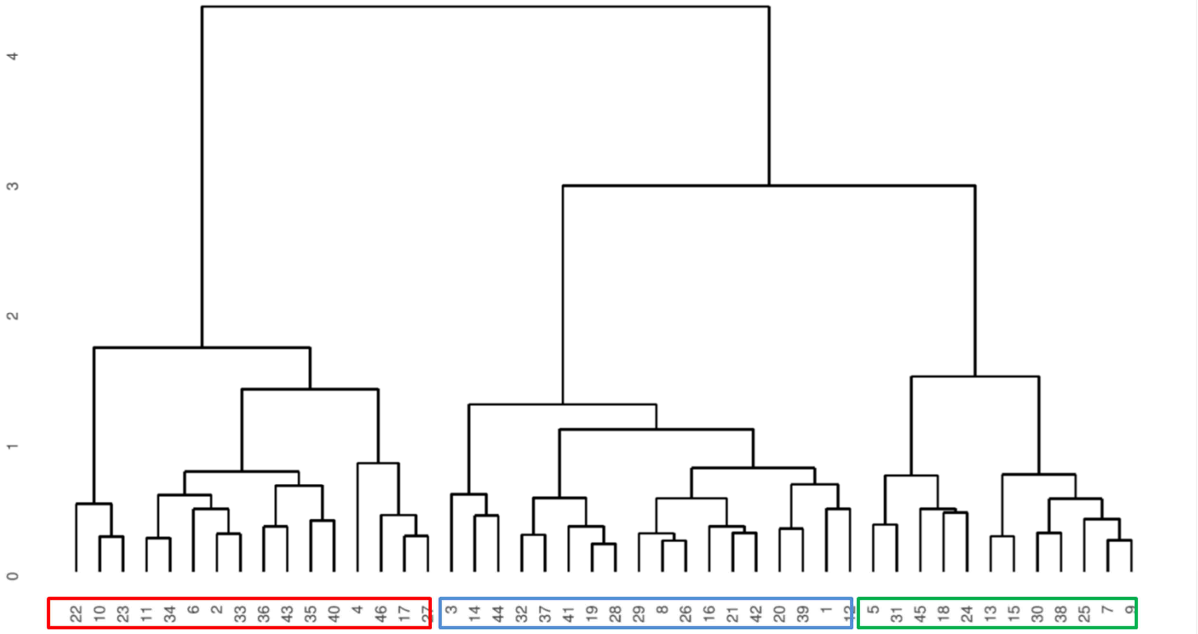


Figure 2.3 Dendrogram distinguishing three major clusters for texture traits of blueberry cultivars, according to their Storage Index.

Texture genetic gain

The texture analysis conducted on 12 putative varieties, among the newest and leading on the EU market, collected from various supermarkets in the UK was added to the original 46 of the experiment. An average timepoint of 10 days storage was considered, in order to obtain a linear regression of the different texture parameters as a function of the year of cultivar release, plotting according to it the mean value of the single parameter.

Some results can be highlighted from this comparison. First of all, an increase in number of varieties available to the market is visible starting from the middle of the ‘80s from different breeding programs, mainly US based. Single berry weight (g) has generally increased over the time, being on average over all the sampled varieties 2,35 g ± 0,67. Although it is generally believed that weight is negatively correlated with firmness, texture comparative evaluation based on linear regression of the parameters maximal force and Young’s modulus show also incremental mean values over the years. On average their respective values were 1,3 MPa ± 0,67 and 3,78 N ± 0,71.

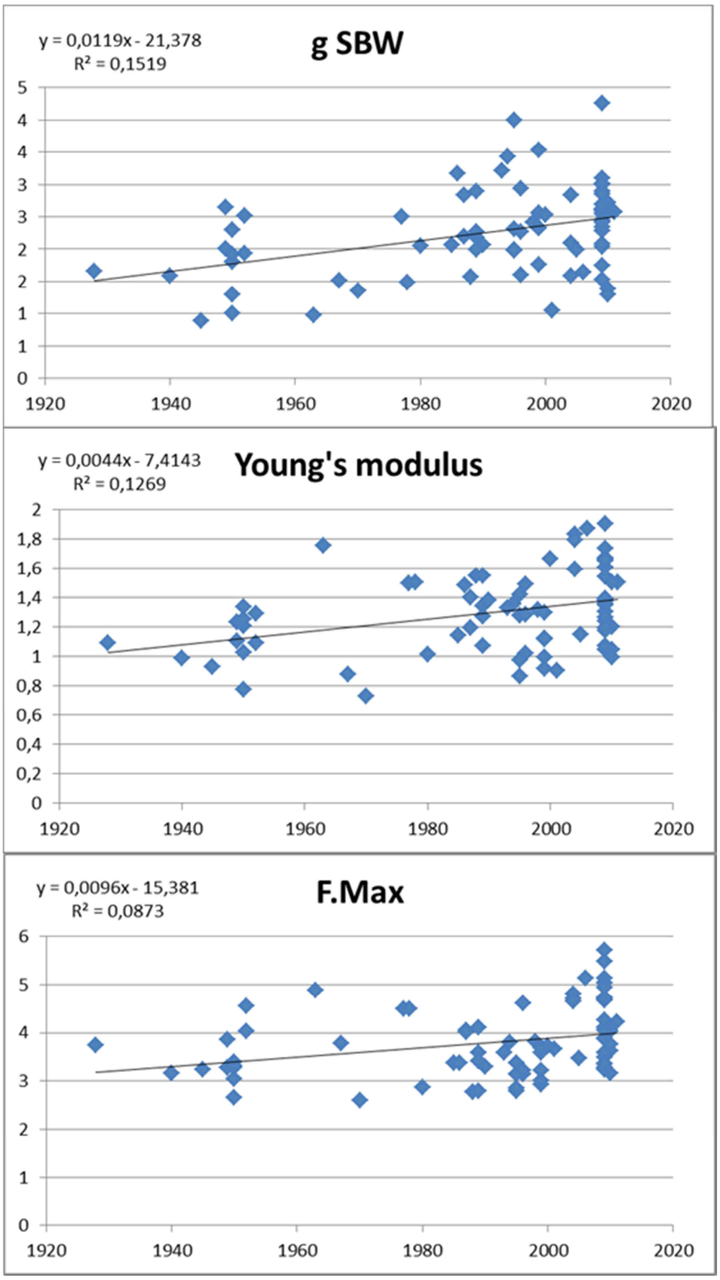


Figure 2.4 Linear regression of the Single Berry Weight (g) SBW, Young's modulus (MPa) and maximum force (N) parameters as a function of the year of cultivar release

High diversity of texture values, but also size, can be observed between older and new varieties, but in general a positive incremental trend that includes the highest values for all the parameters measured is visible in the group of cultivars released after 2000.

This trend is even more evident when advanced selections of the FEM breeding program related to the years 2012-2015 were evaluated. The FEM blueberry breeding program has started the selection process on fruit in 2011, being thus very recent. The first genepool widening approach was designed in 2008-2009 for NHB, while in 2012, SHB hybrids were included in the program.

All the three parameters evaluated showed a gain over the three years of selection, according to mean values that are reported in table 2.3.

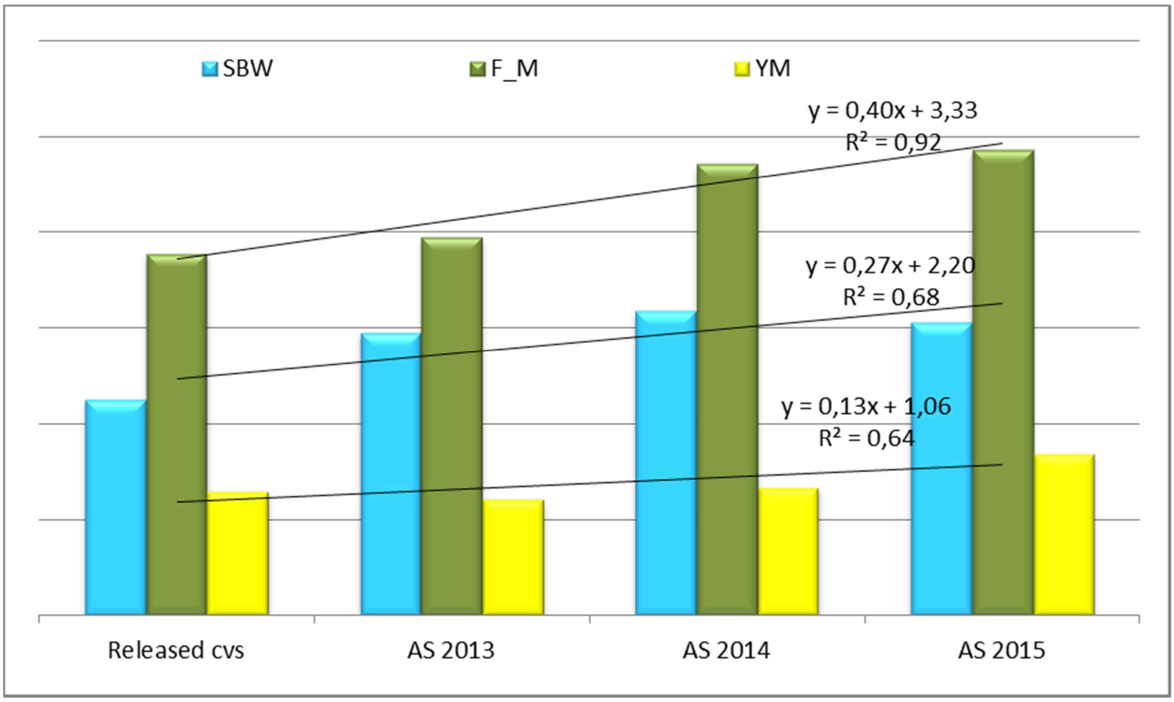


Figure 2.5 Barplot and linear regression of the Single Berry Weight (g) SBW, Young's modulus (MPa) and maximum force (N) parameters as a function of the year of FEM selections (AS) compared to the cultivars released on the market.

	SBW (g)			F_M (N)			YM (MPa)		
	mean		SD	mean		SD	mean		SD
Released cvs	2,25	±	0,67	3,78	±	0,72	1,30	±	0,27
AS 2013	2,96	±	0,33	3,96	±	0,34	1,21	±	0,37
AS 2014	3,18	±	0,58	4,71	±	0,22	1,33	±	0,41
AS 2015	3,06	±	0,32	4,86	±	0,58	1,69	±	0,34

Table 2.2 Mean values and standard deviation of the Single berry weight, maximum force and Young’s modulus for the 46 released cultivars, and four years .

CONCLUSIONS

Improving fruit quality is a major issue for blueberries, strongly affecting marketability, and consequently the economic impact of new varieties. The capacity to choose the most efficient parents to be able to revenue outstanding progenies depends on fruit texture and aroma properties, and on the ability to measure them and use them as selecting tools. To date, fruit texture modifications in blueberry are mainly assessed with end point measurements. In order to overcome these major constraints, we adopted a novel methodology for blueberry (Giongo et al., 2013) for a more comprehensive texture dissection in blueberry. The parameters identified in this work allowed the analytical characterization of texture sub-phenotypes important for blueberry, in particular the texture gradient (elasticity modulus), which has been subjectively estimated by breeders until now using their personal experience. The availability of these objective parameters allows a more precise description of fruit quality, particularly convenient for parents phenotyping and selection in breeding. They can become biomarkers to be used from the very first stages of breeding to segment, according to texture characteristics, into four potential market categories (Fig. 2.6).

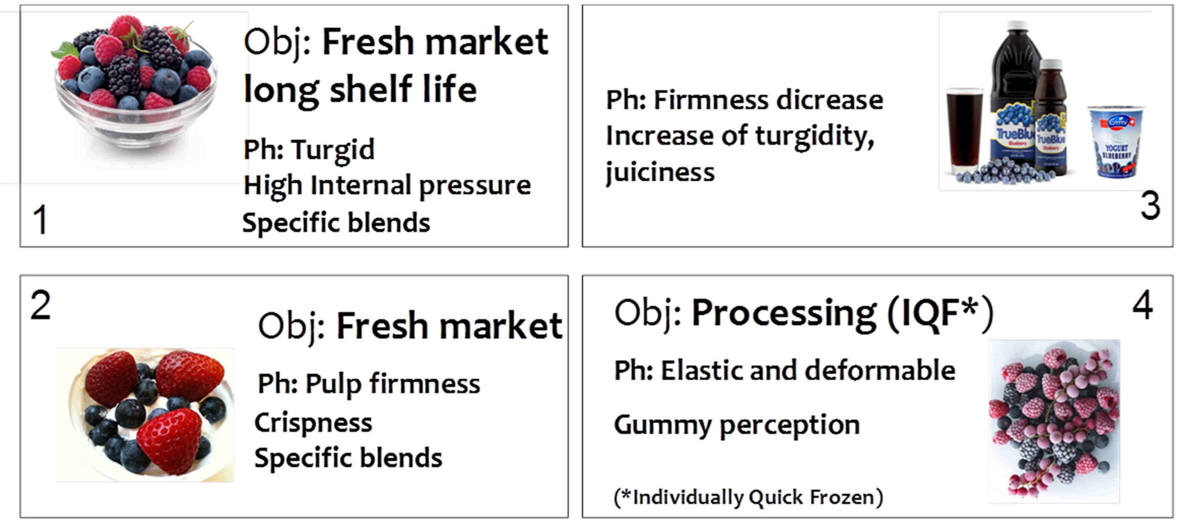


Figure 2.6 Possible first blueberry segmentation into four classes according to texture parameters used as biomarkers in the preliminary stages of the breeding programs.

The genotype-specific and stage-specific texture traits, allow indeed to segment further, thus be more precise in the genetic gain obtained. The application of this technique for phenotyping and its value in the selection process of breeding was shown as a linear incremental regression in the blueberry breeding program.

Fruit quality is certainly not only texture and size, but also other traits were combined in some preliminary analysis. In previous studies, tightly connected with this one (Giongo et al., 2017; Farneti et al., 2017) also the volatilome components was investigated as contributing to flavour on the same cultivars setting. This allow to confirm that a multifactorial analysis for parental choice in blueberry is possible, not only for texture parameters, but also for other connected quality traits.

According to our knowledge, this represents an advancement in phenotyping: texture analysis is a much more reliable and specific analytical techniques than penetrometer. Although still destructive and not high throughput, coupled with other analysis (eg: secondary metabolites) it may allow to choose the best parents for crossing and also to select in the progenies in a relatively short time.

From our results it is evident that even within a specific and not extremely wide germplasm base, the variability present and underexploited until now was great for the traits of interest, thus giving room for future improvements, which are already evident in our breeding programs. This experience show that heritability of some of the identified texture parameters and masses is high, increasing thus the chance to accelerate progress in breeding for these novel traits through an appropriate identification and use of this kind of biomarkers.

Chapter III

Raspberry texture mechanical profiling during fruit ripening and storage

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GL conceived and designed the research, coordinated the experiment, analyzed the data and wrote the manuscript. The chapter refers to the submitted manuscript 2018_732 to Postharvest Biology and Technology, for which permission was granted by the editor. All the co-authors contributed to the research. AM ran the texture samples. MRG contributed to texture analysis. PP sampled fruit and propagated the plants. SP revised the manuscript. FB contributed to the statistical analysis and writing.

INTRODUCTION

In the last decade, the consumption of raspberry (*Rubus idaeus* L.) has drastically increased in the USA, UK and Scandinavian countries and it is foreseen to continue in the next ten to fifteen years (source: Fruit and Nuts 2017). This has gone along with the development of more manageable and storable cultivars (Hafner et al., 2002; Jennings, 2002) together with an improvement of the logistics that allow a constant fruit supply to consumers over the year. However, fruit perishability, mainly due to water loss, morphological fragility, softening, and presence of pathogens like botrytis (Vicente et al, 2009), is still the major problem limiting raspberry marketability. Therefore, high firmness and prolonged shelf life are the two key quality traits of a successful raspberry genotype. Several postharvest techniques have shown to improve shelf life of berries: modified atmosphere with increased levels of CO_2 (Kader et al., 2000; Kumar et al., 2018), irradiation (Wang et al., 2017), rapid fruit cooling, pre-maturation stage picking and use of coatings (Gomes et al., 2017; Mannozzi et al., 2017; Vieira et al., 2016) or gas application of volatile compounds (Tzortzakis, 2007).

Analytical firmness assessment of raspberry is today still challenging due to fruit component heterogeneity (Hall et al., 1999). Raspberry fruit is an aggregate of drupelets, varying by number and seed morphology, which adhere to each other for the drupeolar hair (Jennings, 1988) around a receptacle. Moreover the receptacle may differ for shape, volume/size and type of surface of attachment. The receptacle is completely detached when the ripe raspberry fruit is picked, generating an internal fruit cavity. All these tissues components, in both berries, affect the whole fruit firmness and its evolution during fruit development.

Raspberry firmness measurement has always been either quite simplistic referring to the use of a single point set dimension by penetrometer assessment, or too complex and expensive when it is based on consumer tests or sensory panels. Furthermore, instrumental texture analysis is nowadays a well-established analytical technique in the food industry for evaluating the mechanical and physical characteristics of fruit as well as raw ingredients and finished products (Sato and Yamada 2003; Rolle et al., 2011). These analytical techniques are often preferred to sensory evaluations because they reduce the variability associated with subjective judgments and can be easily carried out also when fruit availability is scarce. Advanced texture assessments have been modelled for fruit with different anatomies, like tomato (Camps, 2017; Schouten et al 2007), apple (Varela et al., 2007), grape (Rolle et al., 2011) and blueberry (Giongo et al., 2013). However, no standard methods are yet available for raspberry. This study aims to develop and validate a novel set of texture parameters for raspberry fruit to be used for advance phenotyping and fruit quality management as predictive and characterizing tools.

MATERIALS AND METHODS

Plant material and fruit sampling

In this study, a total of 29 *Rubus idaeus* L. cultivars of raspberry (Table 3.1), were chosen from the experimental field of Fondazione Edmund Mach - Research and Innovation Centre at Pergine (Trento), located in the north of Italy (Trentino Alto Adige region - 46.0744° N, 11,2334° E, 525 m a.s.l.).

Plants of raspberry were all grown in 7 L pots, under hail net. A fertigation system was applied to guarantee water supply with a commercial fertigation recipe for raspberry. Annual cultivars were developed every year from a new cane, while floricanes were maintained as one year old productive canes. Five plants for each accession were maintained following standard pruning. Berries were harvested manually at the required stage for each experiment, early morning and brought to the laboratory within half an hour picking time. Here, they were put in a refrigerating cell (2 °C) for 2 h and taken out an hour prior to instrumental analysis, maintaining the fruit at room temperature (~20 °C). Homogeneous fruit, without visible external damage or irregularities, were chosen based on size and colour evaluation.

Table 3.1. List of cultivars of raspberry accessions included in the study and respective use in the different experiments.

	Cultivar	Experiment on fruit development (penetrometric test)	Experiment at harvest	Experiment in post-harvest	Compression test	Penetrometric texture test	SI
1	**Allgold**		X		X	X	
2	**Amira**		X	X	X	X	X
3	**Anne**		X	X	X	X	X
4	**Autumn Treasure**		X		X	X	
5	**Caroline**				X		
6	**Cascade Delight**		X		X	X	
7	**Erica**		X	X	X	X	X
8	**Glen Ample**		X		X	X	
9	**Glen Magna**	X					
10	**Heritage**		X	X	X	X	X
11	**HimboTop**		X	X	X	X	X
12	**Imara**		X	X	X	X	X
13	**Joan J**		X		X	X	
14	**Korpiko**		X		X	X	
15	**Kwanza**		X		X	X	
16	**Kweli**		X	X	X	X	X
17	**Malling Juno**		X		X	X	
18	**Meeker**		X	X		X	X
19	**Minerva**		X		X	X	
20	**Octavia**		X		X	X	
21	**Polka**		X		X	X	
22	**Scepter**		X			X	
23	**Regina**		X	X	X	X	X
24	**Sugana**		X	X	X	X	X
25	**Tadmor**	X	X			X	
26	**Tulamagic**		X		X	X	
27	**Tulameen**	X	X	X	X	X	X
28	**Valentina**		X			X	
29	**Versailles**		X	X	X	X	X

To analyse the texture dynamics during ripening, fruit from three different cultivars (Tulameen, Glen Magna, Tadmor) were collected at four ripening stages (Green - G, Pink - P, Ripe - R, Overripe – OR) according to the BBCH-scale (Lorenz et al., 1995), opportunely modified for raspberry .

Twelve raspberry cultivars (Amira, Erica, HimboTop, Kweli, Anne, Heritage, Imara, Tulameen, Sugana, Versailles, Regina and Meeker; Table S1) were subsequently analysed to profile the texture parameters related to fruit ripening and post-harvest (after 3 and 7 d of storage). For postharvest raspberry texture measurements, a batch of pink berries was stored for 3 and 7 d at 2 °C with a relative humidity of 85 %. Other fruit morphology parameters, namely single berry diameter (SBD, mm), single berry mass (SBW, g), single berry height (SBH, mm), drupelets density (N° cm^{-2}) were monitored in order to correlate them to fruit texture parameters. Fruit firmness was preliminarily measured using a digital non-destructive compression fruit tester FirmTechII (UP GmbH, Germany) with a flat 40 mm probe following Bañados et al. (2010), to compare this routine widely methodology to texture analysis as well.

Fruit texture evaluation

Raspberry fruit texture was profiled by employing a texture analyser (Zwick Roell, Italy), using two different methodologies: a destructive penetration test and a non-destructive compression test. All the operative conditions, acronyms and formulas are resumed in table 1.

Table 3.2 List of the main mechanical parameters related to the texture raspberry profiling and relative characteristics applied through penetrometer, compression (TPA) and relative indexes on the set of cultivars at different stages and in post-harvest.

Mechanical parameters	General description of the parameters	Unit	Curve	Acronym	Reference
Max force	Maximum force value recorded over the probe's travel	N	Force-deformation	FMax	Giongo et al., 2013.
Min force	Minimum force value recorded over the probe's travel	N		Fmin	
Final force	Force measured at the end of the probe's travel	N		Ffin	
Area	Area underlying the mechanical profile	N mm		Area	
Deformation at maximum force	Computation of the maximum force associated with the curve on its whole length	%		Def_FMax	
Deformation at minimum force	Computation of the minimum force associated with the curve on its whole length	%		Def_Fmin	
Gradient	Young's modulus or elasticity modulus, computed as ratio between stress and strain	MPa		G_YM	
Slope 1	Slope of the first cycle, called Young's modulus of elasticity.	N mm^{-1}	Force-deformation	SF1	Letaief et al., 2008; Maury et al., 2009; Rolle et al., 2011.
Slope 2	Slope of the second cycle of compression	N mm^{-1}		SF2	
Work 1	Work associated with H1, which is the energy of the system, at the first compression.	J		W1	
Work 2	Work associated with H2, at the second compression.	J		W2	
Hardness 1	Hardness, maximal force expressed at the first compression, needed to attain a given deformation.	N		H1	
Hardness 2	Hardness, maximal force expressed at the second compression	N		H2	
Cohesiveness	Strength of the internal bonds making up the berry body, calculated through the formula BCo=W2/W1	-	Force-time	BCo	Rolle et al., 2011
Gumminess	Berry gumminess (BG) is the force necessary to chew a semisolid food until ready for swallowing and it is calculated as BGu=BCo x H1	N		BGu	
Chewiness	Berry chewiness (BCh) is the energy needed to chew a semisolid food until ready for swallowing and it is calculated as BHxBCoxBS	mJ		BCh	
Springiness	Springiness is the measured distance recovered by the sample during the time between the end of the first bite and the start of the second one.	mm		BS	

Penetration test

The penetration texture analysis outlined a mechanical force displacement using a 5 kg loading cell and a cylindrical flat head probe with a diameter of 4 mm entering into the berry flesh from the sagittal side. The mechanical profile graph was based on two fundamental variables: force (N) and distance (strain, %). Mechanical profiles were acquired with a resolution of 100 points per second with the following instrumental settings: test speed of 300 mm min^{-1}, post-test speed of 1000 mm min^{-1}, auto force trigger of 2 g and stop plot at target position. Each berry was penetrated until a 99 % penetration strain.

The penetration test was preliminarily applied on three cultivars at four different developmental stages to set the main parameters, which were designed based on the force displacement profile: maximum force (FMax, N), minimum force (Fmin, N), final force (Ffin, N), area (Area, N mm), gradient parameters indicating the Young's modulus (G_YM, MPa), deformation at maximum and minimum force (Def_Fmax and Def_Fmin both expressed in %).

The texture profiling through penetration was carried out on a set of 27 cultivars, listed in S.1, assessed at harvest. Twelve of these cultivars (Table S1) were evaluated also after 3 and 7 d of cold storage. Based on these results the storage index (SI) was computed using the formula (proposed by Giongo et al., 2013), SI = $\log_2(Ti_{PH} / Ti_H)$, where Ti_H is the value of the 'i' texture parameter measured at harvest, and Ti_{PH} is the value of the same parameter measured after cold storage. Positive SI values indicate a texture sub-trait enhancement, whilst negative values point to a loss of textural performance during storage. An SI equal to zero means stable maintenance of the texture sub-traits under investigation.

Compression test

The non-destructive compression method based on a double cycle test (Deng et al., 2005, Letaief et al., 2008; Maury et al., 2009) was conducted on 24 cultivars (Table S1) on 10 to 30 fruit per variety. The waiting time between the two cycles of compression was 5 s. Berries were compressed by using two parallel plates (platform and flat probe) up to 2 mm on the berry height. The diameter of the stainless flat probe was of 75 mm. Test speed was set at 300 mm min^{-1}; point of application of the charge of the cycles was 20 % over the deformation; cycle speed was fixed at 100 mm min^{-1} with the test ending at 99 % deformation. Trigger force was set at 0,1 % with a nominal force of 2 g and a pre-charge of 0.05 N. Distance between tools was 40 mm. All the curves were acquired and analysed for different attributes as derived by grape studies Maury et al. (2009) and Rolle et al. (2011). The parameters derived from the profile were the force/strain curve, namely berry hardness associated with the first compression, corresponding to the maximal force (H1), and with the second compression (H2); the work associated with H1 (W1) and with H2 (W2), which is the energy of the system; the slopes of the first (SF1) cycle, called Young's modulus of elasticity and the slope of the second cycle (SF2). Cohesiveness and gumminess, corresponding to the ratios BCo= W2/W1 and BG = BCo x H1 respectively (Breene 1975) were also considered. In addition, berry chewiness (BCh), was derived using the formula BCh=H1 x BCo x BS. Berry springiness (BS) was calculated by measuring the distance of the detected height of the product on the second compression.

Data analysis

The data acquisition carried out on the mechanical profiles was operated by the software Exponent v.4 (Stable MicroSystem, Ltd., Godalming, UK) provided with the Zwick instrument. With the same software a macro instruction was also compiled to automate the parameter extraction from the profile to excel.

Data analysis was performed with R.3.4.4 software using internal functions and the external packages “mixOmics” and “heatmap3” for multivariate statistical analysis, “corrplot” for the Pearson’s correlation analysis, and “ggplot2” for graphic representations.

RESULTS AND DISCUSSION

Texture during fruit development

Raspberry development during fruit growth and ripening mainly involved changes in berry size and colour (data not shown). Likewise, texture profile considerably changed through the four selected stages (G, P, R, OR), mostly for force values (Fig. 1A). A great force drop is evident during the evolution from the green to the overripe stage, as expected using a penetrometric analysis (Fig. 3.1A).

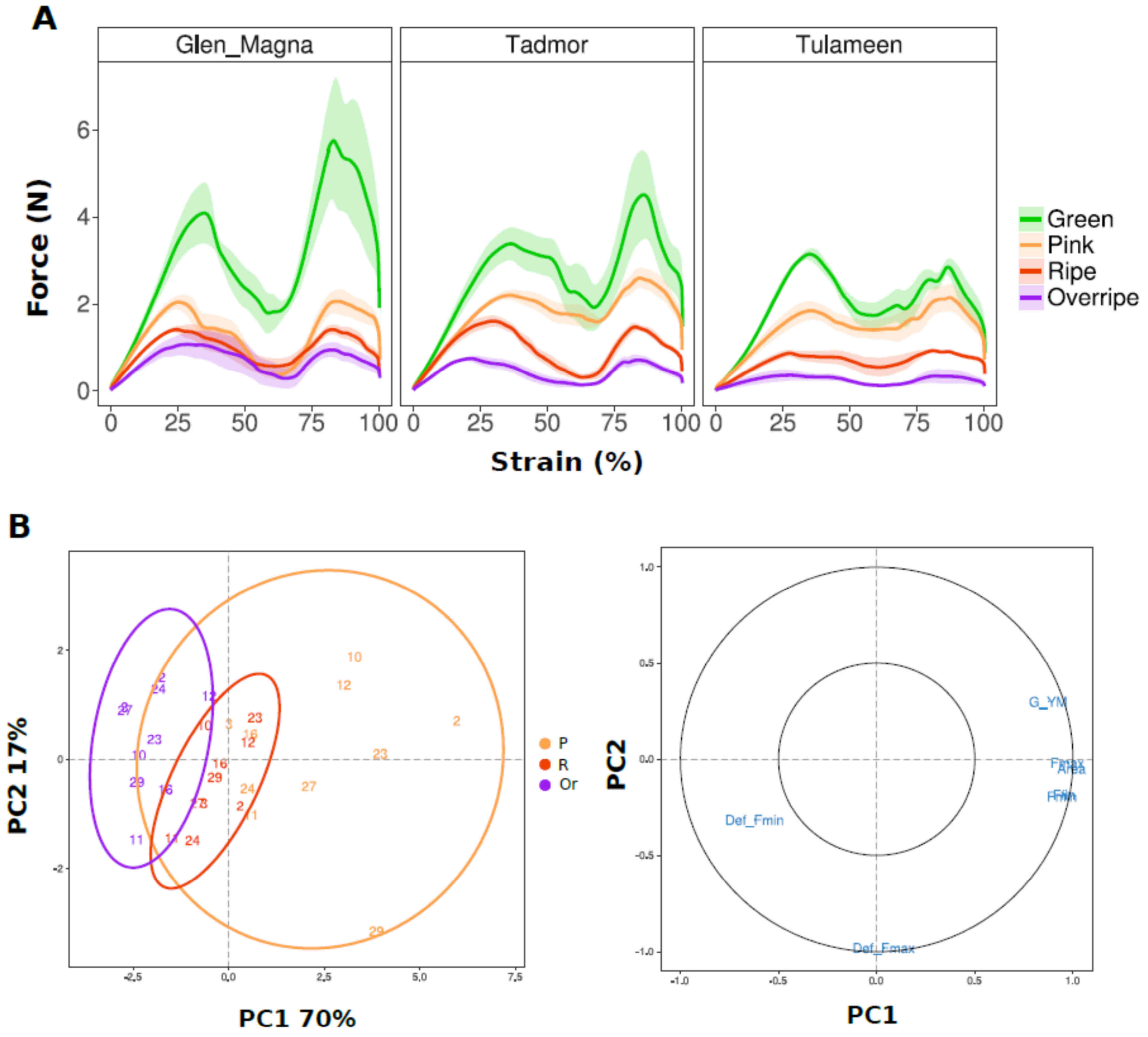

Fig. 3.1: A. Raspberry development during fruit growth and ripening through four selected stages (Green, Pink, Red, OverRipe) for three cultivars (Glen Magna, Tadmor and Tulameen). Force (N) in the y-axis and strain (%) in x-axis. Error bars represent means ± SE (n = 5). **B.** Principal Component Analysis representing the distribution of the raspberry cultivars during fruit growth and ripening through three selected stages (Pink, Red, OverRipe) and relative loadings of the texture parameters. Ellipses indicate the 95 % confidence.

The force–displacement curves (Fig. 1A) show no major fracture, but a continuous flow drawing two major peaks/waves. When the probe punctures the raspberry fruit – differently from other fruit like blueberry or grape – it does not fracture the epidermis but cell packs with the inferior tissue layers, producing a double wave more than a double peak. These results involve all the forces contributing to opposing resistance by the two layers of drupelets with the receptacle cavity in between. This can suggest that skin strength may not markedly affect raspberry texture , at least using this method, and on the contrary that the receptacle cavity has a great influence on textural profile. The green stage is characterized by high values of the Area (257,78 N mm ± 87,5), F_Fin (3,85 N ± 2,31), FMax (3,57 N ± 1,05), G_YM (0,45 MPa ± 0,15) and FMin (1,74 N ± 0,93), while Def_Fmax (7,07 % ± 1,11) and Def_Fmin (11,33 % ± 1,66) are not significantly different than at the other stages.

Area, F_Fin, FMax, Fmin and G_YM decrease their respective values from the green to the overripe stage, while Def_FMax remains slightly stable and Def_Fmin slightly increases, due to a general softening of all the contributing tissue layers of the fruit and disassembly of cell walls.

Differences among ripening stages are significant when different cultivars are analysed. PCA shown in Fig.S2 describes a high variation (96 %) when the seven penetration parameters are used to describe the texture profiles of raspberry cultivars at different ripening stages.

The direction of the loadings distinctly separates according to all the forces, and PC1 explains 90 % of the variability, grouping all the green samples in the second and third quadrants from the other stages in the two opposite quadrants. When the green stage is excluded for scaling reasons in the results evaluation, the patterns are less evident: the PCA in Fig. 1B shows the groupings defined at pink, red and overripe stages for the three selected cultivars Glen Magna, Tulameen and Tadmor. The total variation of these data is explained up to 87 %. PC1 (70 %) is mostly defined by forces parameters, Young's modulus and deformation at minimum force, while PC2 (17 %) explains the variation based on the deformation at maximum force. Although the parameters contribute to distinguish less clearly pink, ripe, overripe berries, the two groups of ripe and overripe fruit are more evident, while pink fruit tends to overlap and widely spreads over the four quadrants of the PCA. Pink stage harvest is a common management practice to prolong shelf life in raspberry, but these results indicate very high cultivar specificity thus the development of the textural faith in the stages following the pink one is highly dependent on the genotype. Some cultivars, such as Amira, Regina, and Heritage, are very firm at the pink stage, but they drop dramatically as the ripening process advances. Other cultivars, like Versailles and Kweli, are instead more stable during ripening, although the initial force is lower (pink stage). The two groups have a different genetic background thus suggesting a genetic control of the trait that might be related to what is reported in other Rosaceae crops like strawberry (Mathey et al., 2017) and apple (Cevik et al., 2010; Ben Sadok et al., 2015).

Texture comparison through penetrometric at harvest, post-harvest and storage index

The texture penetrometric scores at harvest were registered on 10 to 30 berries for each of the 27 considered cultivars. The first two principal components of the PCA, based on 7 descriptors used in the penetrometric texture analysis at harvest, accounted for 73 % of the total variation in the data (Fig. 3.2).

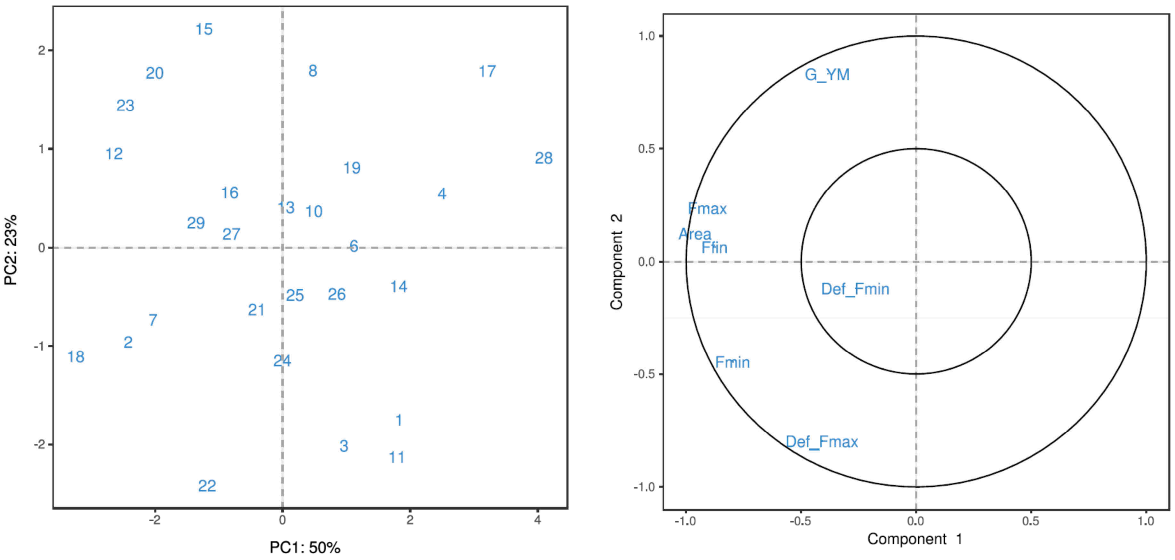

Fig. 3.2. Principal Component Analysis representing the distribution of the raspberry cultivars at harvest and relative loadings of the texture parameters through penetrometric test.

The 27 cultivars are spread over the four PCA quadrants, indicating clear different textural patterns and high variability among them. In all the representation and discussion of the results, the first PCA quadrant indicates positive values of PC1 and PC2, the second negative PC1 and positive PC2, the third negative PC1 and PC2 and the fourth positive PC1 and negative PC2. The distribution of the varieties all over the four quadrants allows to group at least according to the loading directions. PC1 accounts for an explanation of 50 % of the variability among cultivars and defines those that show mainly high values of FMax, Area, FFin and FMin, in the second and third quadrants, from those that show an opposite trend and are spread over the first and fourth quadrants. Minimum force direction in the third quadrant is mainly related to the receptacle cavity of the fruit, which represents an anatomical barrier to texture composition. The larger and profound the hollow, the more susceptible to decay is the raspberry. When we measure minimum force via texture analysis, the highest is FMin the smaller the hollow, thus the highest the resistance of it to penetration. Imara, Amira, Regina, Meeker belong to this group. The penetrometric texture measurement explains in our database about 50 %, but other 23 % of the variability is explained by PC2, which is mainly due to the loadings of the Young's modulus and deformation at maximal force, which separate the cultivars accordingly. If only the maximum force were to be considered, as using a single point penetrometer, raspberry genotypes would have been discriminated only based on the force values, therefore losing the information about texture deformation. Thus, the introduction of other parameters, namely the Young's modulus and deformation, than simple firmness or hardness, allows for a more precise and complete texture analysis.

Texture dynamics in post-harvest for twelve cultivars at 3 and 7 d of cold storage and is cultivar-dependent. Analysing the single parameters over the two storage timepoints, the attitude of the single cultivars to change their textural components in a cultivar specific manner is clear and significant (Fig. 3.3).

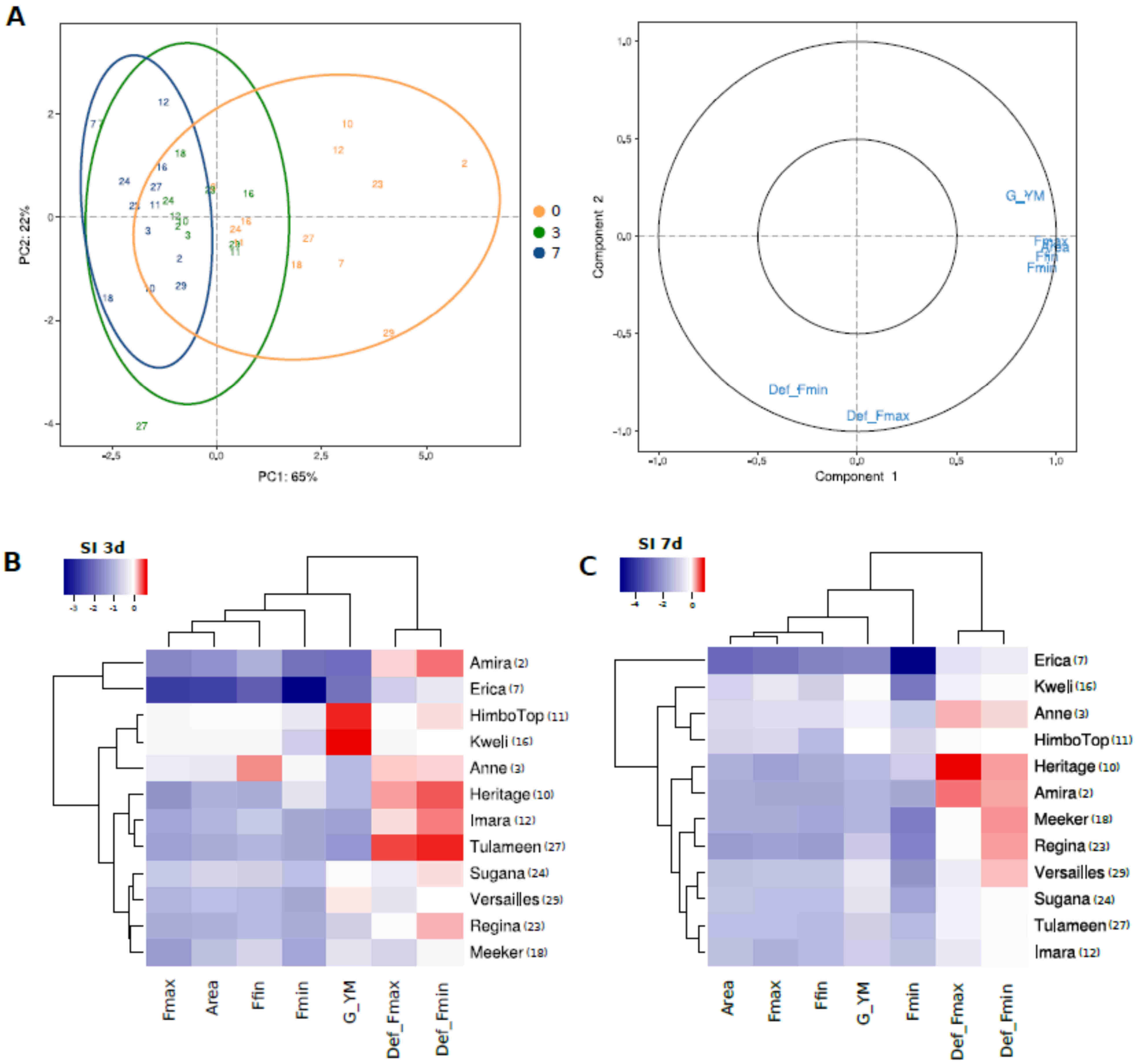

Fig. 3.3. A. Principal Component Analysis representing the distribution of the raspberry cultivars in post-harvest and relative loadings of the texture parameters through penetrometric texture analysis. Ellipses indicate the 95 % confidence. **B.** Resulting heatmaps of the texture parameters at 3 and 7 d of storage contributing to the differentiation of each of the cultivars and calculated as storage index.

The storage index (SI) provides information related to the magnitude of the variation for each parameter, rather than an absolute value. The PCA plot (Fig. 3.3A) explains 87 % of the total variation of the texture in this set of cultivars. The loadings of Young's modulus are consistent with the situation observed at harvest, where this parameter is orthogonally oriented with the fruit resulting deformation at maximum and minimum forces. These latter seem to be less independent in post-harvest, but still significant, indicating as expected a general decay of the fruit, due to internal tissue layers resistance, indicated by the nearness of the Young's modulus to the other forces, however, still resolutive. The two parameters related to deformation (Def_Fmin, Def_FMax) maintain their vector orthogonal independence, denoting their value in dissecting the variance among cultivars. The PCA analysis plot shows different groups of varieties according to the parameters used to analyse them: Himbotop, Anne and Kweli show higher values of the Young's modulus and thus higher turgor and a more resistant skin and mesoderm tissues. Versailles is positioned in the direction of higher deformation and forces, implying a good attitude to storage, mainly due in this case to more internal tissue layers. Erica is plotted on the opposite quadrant, reflecting a less firm and more elastic attitude of the same layers. The distribution cloud includes several varieties that show intermediate values for the texture components.

The storage index (heatmaps of the Fig. 3.3B) clearly indicates important variation for all the sub-traits, revealing thus specific texture profiles clearly patterned for post-harvest attitudes. After 3 d of storage, positive values of SI are more related to the deformation parameters and partially to the Young's modulus. At 7 d of storage, the factors decrease progressively from positive to values close to zero. Generally, all the parameters related to forces have a negative dynamic. When the cultivars dynamics are evaluated, Erica shows for all the parameters negative values, while Himbotop and Kweli show positive values of the Young Modulus indicating a major suitability to preserve a favourable texture performance over three days of storage and strengthening the importance of knowing this parameter together with the others that contribute to raspberry texture phenotyping. All the varieties show a general decrease of the values after seven days of cold storage but with a significant genotypic quali-quantitative specificity, being deformation at maximum force the only parameter that has a positive evolution or no change.

This experiment underlines the importance of dissecting texture in its major components to monitor precisely the dynamic of evolution during post-harvest of a cultivar. This can affect cultural management: from a texture point of view, in fact, the two distinct patterns allow on one side to pick ripe fruit, which have a lower piking rate per hour and still can store adequately. On the other side, this allows to be able to choose among cultivars that do or do not drop the texture quality, which would indicate a positive or negative attitude for storage and shipping. In addition, when raspberry is harvested pink – a very common practice for a number of fresh market growers – the main risk is that instead of following a physiological maturation process, which can positively contribute to fruit quality through an increase of sugars and aromatic compounds, this process is interrupted and only degenerative cellular processes are involved.

Thus, the cultivar and stage specific texture dynamics can have important commercial implications related to sorting but also influence the pre-harvest management that goes from the cultivar choice to the picking stage.

In addition, another output is represented by the experimental requirement of testing the widest genepool as possible to infer the highest variability of the texture parameters, before their validation. For the experiment run at harvest and post-harvest, some closely genetically related cultivars were used among the others. For instance, Tulameen is one of the parents of Amira, Regina and Sugana, while Imara, Kweli, Kwanza and Versailles belong to a different genetic pool: the grouping related to textural profiles is evident in the results both at harvest and post-harvest. Firmness has been shown to have a genetic control in tomato (Causse et al., 2003), in blueberry

(Giongo et al., 2017), in apple (Chagnè et al., 2014), thus it can be hypothesised that also in raspberry there can be a similar genetic control. When the trait is phenotypically characterized through a single data point measurement, like it has been for firmness over the years, the measurement per se includes several other parameters. They differently contribute to the profiling and the resulting dynamics is the contribution of different individual cardinal phenomena like cell size and number or tissues distribution in the fruit anatomy. Thus, dissecting texture components into different specific variables can certainly help to better phenotype and genetically associate the trait. When the dataset of genotypes is enriched, also the validation of the parameters acquires importance in the improvement of the profiling methodology or profiling. The cultivars' set used in this study includes a wide genetic variability for a crop that is however highly inbred. The set included among the most successful cultivars for the fresh market, like Tulameen, Kwanza, Erica and Versailles, for which – in addition to the size of the fruit, the contribution of the external anatomical layers of the fruit can influence texture profiling and likely perception.

The cultivars that show intermediate Eigenvalues in post-harvest, like Sugana (Fig 3B) compared to the other two previously described groups of cultivars show a gradient of parameters among the two, indicating a limited but still variable and cultivar specific potential for storage. This attitude might be related to the structure of the endocarp, which in raspberry is stratified and consists of two layers of elongated thick-walled sclereids. The inner layer is made by sclereids that form transversal rings bounding the ovary and oriented perpendicularly to the longer axis of the pyrene, and the outer layer, in which sclereid cells run at a right angle to the inner layer of sclereid cells, parallel to the longer axis of the pyrene.

Texture comparison through double cycle compression

Deformation and elasticity of the external tissue layers via the penetrometric texture analysis appeared to be crucial to completely define raspberry texture profile. To be more precise in the characterization of the different parameters and to apply a method that could mimic what happens when the fruit is touched or eaten simulating a double cycle of compression to define the deformation undergone in the fruit, the TPA methodology, applied in several other crops like grape (Maury et al., 2009), pear (Pham et al., 2017), mango (Imran Al-Haq et al., 2004) and apple (Gòmez et al., 2011) was thus complementarily and comparatively chosen. Raspberries were compressed twice in their equatorial position and the initial trials helped to set the limits of the experimental design. In this study, strain is expressed as deformation (mm), and Force/Strain curves were determined with eight mechanical parameters (Table 1). These were hardness associated with the first compression, corresponding to the maximal hardness (H1) and the 2nd compression (H2), work associated with H1 (W1) and with H2 (W2) calculated with the minus square method, the slopes of the first (SF1) and the second compression (SF2) calculated in the linear zone prior rupture (modulus of elasticity). Fig. 3.4 shows two typical profiles of the force/strain curves obtained during two compressions of grapes, one based on deformation, the second on time for four representative cultivars (Anne, Tulameen, Caroline and Kweli).

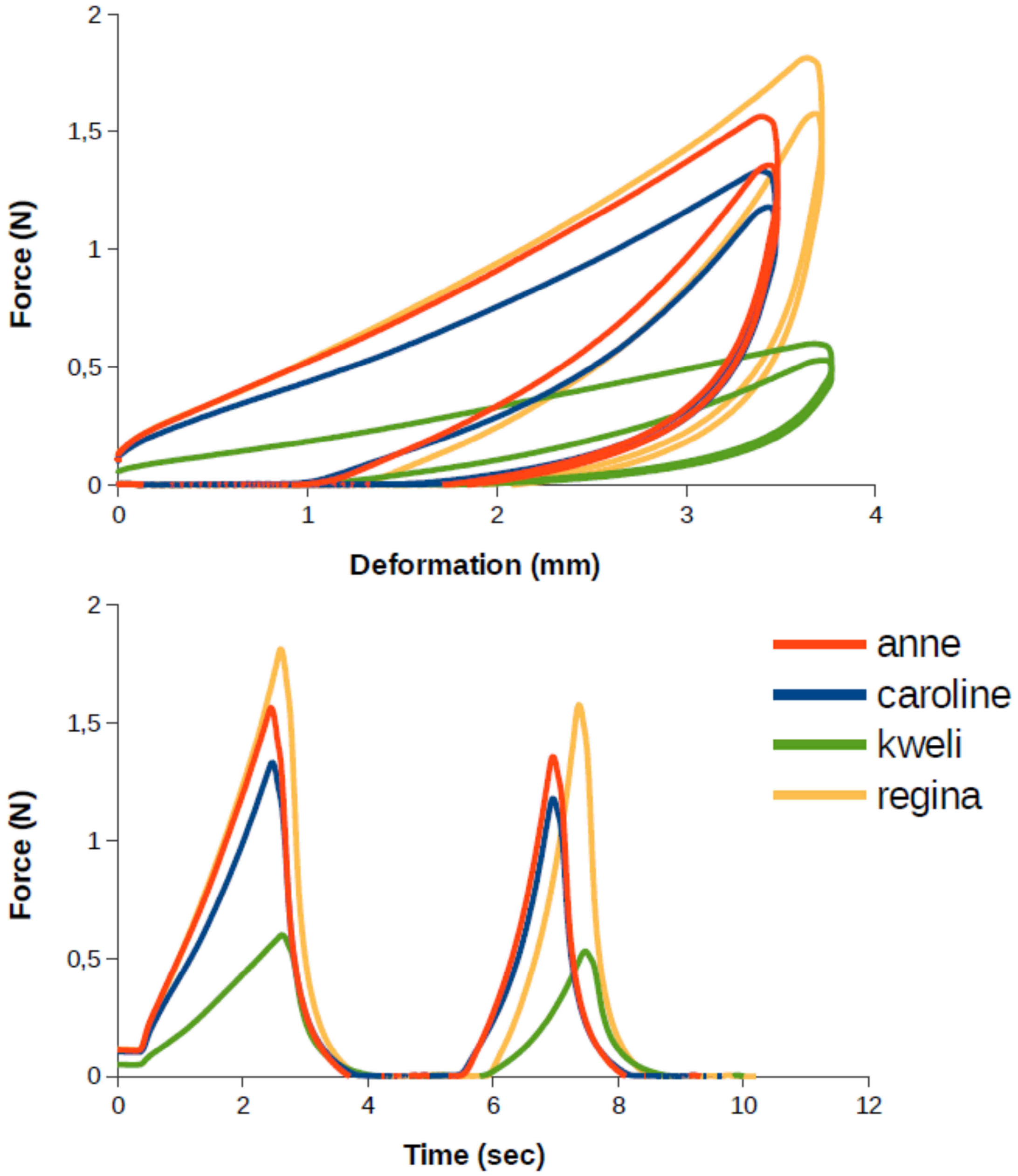

Fig. 3.4. Graphical representation of the double compression curves for four cultivars through compression test expressed by the profile "force (N) in the y-axis and deformation (mm) in x-axis" in the first chart and "force (N) in the y-axis and time (sec) in x-axis" in the second.

Cultivars differences are related to all the parameters: H1 is the maximal hardness that corresponds to the force needed to compress the berry for 20 % of its diameter and W1 is the energy needed to produce this level of compression. Caroline shows the highest values for H1 and Kweli the lowest. H2 and W2, maximal hardness and work needed of the second cycle respectively, are lower for all the cultivars, indicating that the berry does not come back to the initial shape. In the same way also the slopes, that indicate the elasticity of the fruit during the first (SF1) and second (SF2) cycle of compression, show a decrease in values and varietal specific differences. This suggests that destruction of cells, cell walls and tissues packing without skin breaking may be occurring, as in Perkins-Veazie et al., 1992. The compression original parameters H, W and SF related to the first and second cycles, are graphically drawn in Fig. 4 for four representative cultivars. The resulting PCA plot in Fig. 3.5A, accounts for 97 % of the variation in the data and the resulting distribution of the 24 cultivars analysed with different texture profiles over the four quadrants according to the compression parameters.

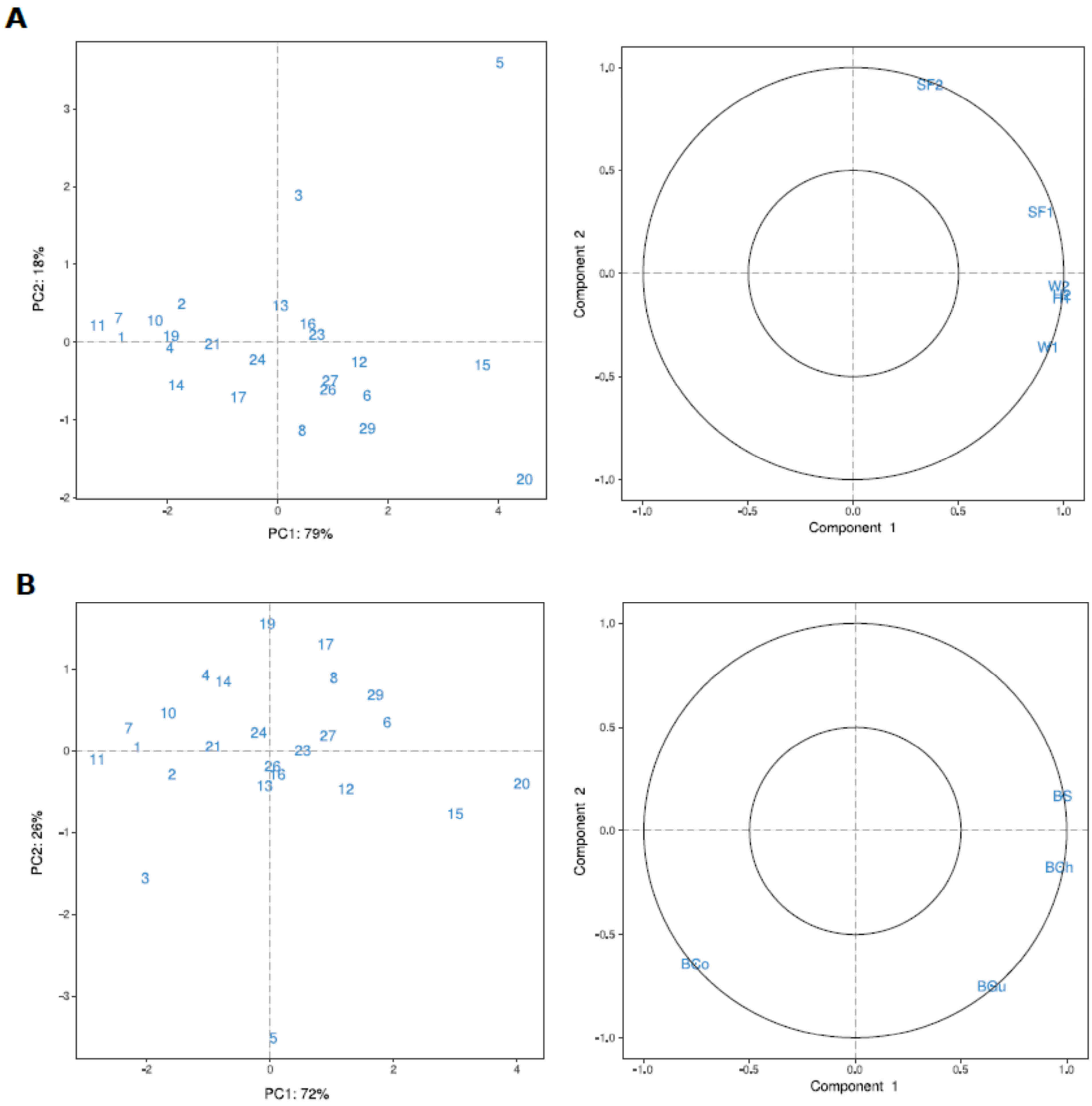

Fig. 3.5 A. Principal Component Analysis representing the distribution of the raspberry cultivars at harvest and relative loadings of the texture parameters through compression test. **B.** Principal Component Analysis representing the distribution of the raspberry cultivars at harvest and relative loadings of the texture indexes calculated after compression test.

PC1 mainly expresses variability in the forces/hardness (H) and work (W). The second and third quadrants include varieties that plot in a cloud characterized mainly by low values of W1 and W2, which are the energy of the system both at the first and second compression, as well as H1 and H2, which represent hardness, thus the maximum force expressed to attain the given deformation. PC2, which contributes for the remaining 18 % of the variability, mainly differentiates the cultivars according to their elastic properties (SF2 in particular). Caroline and Anne are clearly positioned in the first quadrant, which is characterized by high values of SF2, the slope of the second compression or Young's modulus expressed in the second cycle, while Glen Ample, Octavia and Versailles show an opposite attitude for this parameter.

In order to implement the characterizing nomenclature, a number of indexes, applied also to grape, which describe gumminess, cohesiveness and springiness were derived to better explain raspberry texture. Being stemmed by the original TPA compression parameters, they do not change dramatically the resulting distribution of cultivars. The variation explained in the PCA (Fig. 5B) is always high (98 %), but the indexes allow to better define the textural composition of the cultivars according to more comprehensive and accepted terms. The first quadrant is mainly characterized by berry springiness and here belong Versailles and Cascade Delight. The PC1, in addition to BS, shows high loadings of chewiness BCh, for which Octavia and Kwanza express the highest values. Gumminess plots in the fourth quadrant, independent both from springiness and from cohesiveness. Caroline is a cultivar that shows high values of both BGu and BCo, showing the highest values of PC2. Cohesiveness vector is loaded in the third quadrant and the cultivar Anne is the most representative of this parameter.

Correlation of parameters and descriptors

Morphological and mechanical texture measurements provide important characterization of the berries and it is crucial to know if and how the descriptors and parameters used and developed are linked. Therefore, a correlation matrix was calculated for each individual parameter and results are shown in Fig. 3.6.

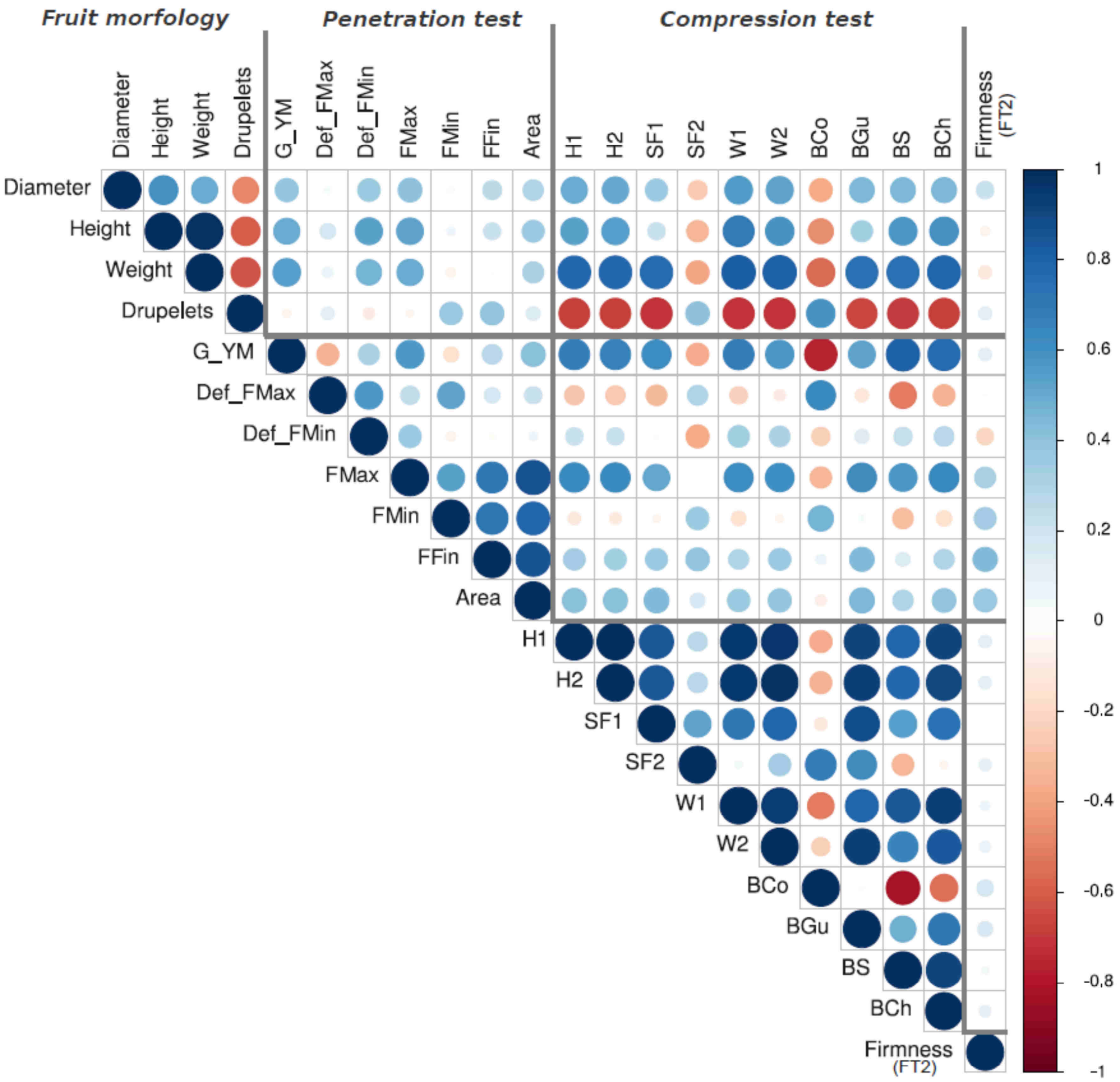

Fig 3.6. Correlation matrix built on 22 texture and morphological raspberry descriptive parameters. Positive correlations are displayed in blue and negative correlations in red color. Color intensity and the size of the circle are proportional to the correlation coefficients.

In other berries like blueberry (Giongo et al., 2013) and grape (Rolle et al. 2011), the skin is a proper textural barrier, thus specific measurements of the Young's modulus can be tailored and information on the epitelial and mesodermic layers can be deduced. In raspberry, however, the skin seems to be not significantly contributing in terms of textural profiling, at least when penetrometric techniques are applied. A comparison of 22 texture mechanical parameters, including all the penetrometric and compressive ones, was conducted on 892 fruit of different cultivars both with the penetration textural method and the TPA, to keep the significant ones and drop all the others. The parameters were related to penetration (7), compression (10) and one related to the measurement conducted through the FirmTechII. With the penetrometric texture measurement, the elastic parameter referred to the Young's modulus, that considers the epidermis and mesodermic contribution of forces applied, results as being the most robust to profile the elastic properties of raspberry. Between the two methodologies applied, according to the correlation matrix derived for all the parameters, this parameter can be either significantly discriminative through compression (SF1 and SF2) and through penetration (G_YM). The main forces parameters compared with the two techniques seem to be equally resolutive: H1 and H2 correlate with maximum force respectively by 64 %, while they did not significantly correlate with the deformation at maximum and minimum forces, indicating a less discriminating potential to profile more internal tissue layers and the receptacle cavity. Lastly, it is interesting to highlight that none of the parameters identified is significantly correlated with the firmness measured by FirmTech 2. The lack of sensitivity of this measurement, already noticed also in other studies when applied to raspberry and other berries (Hall et al., 1999; Li et al., 2011), reinforces the need to better tailor the methodology to measure raspberry firmness.

Both penetrometric and compression parameters were differently correlated with the fruit morphological characteristics. Fruit size (height and weight) is more significantly correlated with compression measurements of the external and internal layers, while only weakly or none for drupelets with the penetrometric texture analysis. The number of drupelets is significantly negatively correlated with all the parameters used for the compression test, except for SF2 and cohesiveness, and all the relative indexes derived, namely gumminess, chewiness, and springiness. However, the number of drupelets was not correlated with any of the penetrometric indexes, underlining the importance of using both methodologies to dissect all the components for fine phenotyping. The correlation of the number of drupelets with the morphological parameters is negative both with the single berry weight (r=-0,61) and height (r=-0,62). These results follow the study of the anatomical structure of raspberry proposed by Williamson et al. (1994), according to which a high number of drupelets indicates a major structural adhesion to the receptacle, which results thus as a stronger structure and suitability to picking or machine harvest.

CONCLUSIONS

Texture analysis is a highly resolutive technique when applied to food and to fruit with difficult anatomical structure such as raspberry. The two methods applied in this study, penetrometer and compression, can be used as suitable technologies to objectively monitor berry texture during development, at harvest and after storage, in order to identify cultivar differences and predict the attitude to postharvest storability.

A relationship among texture characteristics was elucidated here to estimate their magnitude and directions. A high variation was explained among raspberry cultivars: differences among genotypes were observed at all stages analysed, showing a significant cultivar dependent pattern at harvest and storage.

The choice of texture analysis method depends on the objectives of the measurement and phenotyping. The two methodologies, one destructive and the other non -destructive, should be combined to obtain more reliable texture phenotyping. Firmness or hardness also in raspberry is more nuanced than a simple peak force metric, and this is demonstrated by this study, where the textural effect of external – skin and mesoderm – internal and cavity tissues was defined by the different parameters set in the different analyses.

The Young's modulus (or modulus of elasticity) is another important strength parameter that texture analysis can measure, used in predicting the deformation that occurs in fruit and vegetables under loading. This parameter is a measure of the inherent stiffness and resistance to elastic deformation and mathematically expresses the fruit’s tendency to be deformed elastically under compression. Raspberry fruit is characterized by a very low Young's modulus, but it shows significant genotypic and ripening stage differences. Raspberry changes fruit shape considerably during its development and is thus liable to deform by a substantial amount under compressive loading. The ability to clearly identify this parameter such as Young's modulus which is highly informative of external damage symptoms for mechanical deformation, can help to adopt different strategies to predict shelf life attitude and to reduce postharvest losses. Furthermore, it improves specific fine phenotyping, which might positively contribute to genetic association studies towards breeding studies.

Texture analysis on raspberry must be further widened: sensory analyses need to be coupled to the produced results in order to advance the information, as well as genetic and genomic studies on these traits. Microscopy and physiological data can also reinforce the quality texture profiling, as well as being able to monitor other quality parameters here not included, like juiciness, or to develop alternative non-destructive methods like acoustic and optical approaches (Chen et al., 2013) that can provide 'real-time' or 'on-line' texture measurement for fresh and processed foods.

In conclusion, due to the current lack of international standards for raspberry texture measurements and fine phenotyping, this study on raspberry texture analysis can contribute to define parameters settings able to profile, trace and compare quality components on this berry category using the same or similar instruments.

Chapter IV

Strawberry texture profiling during fruit ripening and storage: a key to implement breeding for fruit quality

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GL conceived and designed the research, coordinated the experiment, analysed the data and wrote the chapter. All the authors contributed to the research. JPB with his work thesis ran some of the texture samples, data analysis and writing. PM and MA contributed to texture analysis and morphological data. PP sampled fruit and propagated the plants. CN ran the non-volatile secondary metabolites. PS revised the chapter. BF contributed to the statistical analysis and writing.

INTRODUCTION

Strawberry (*Fragaria* x ananassa Duch.) is globally the most consumed berry due to health benefits that make it a superfood, due to a wide range of adapted varieties to different climatic regions and to the quality of the fruit (Appleton et al., 2016).

In 2016, the global strawberry market amounted to 9.2M tonnes, being consumption and production quite diverse per area. The countries with the highest consumption were in 2017 China (41%), the U.S. (16%), Egypt (5%), Turkey (4%), Mexico (4%) and Germany (3%), together comprising almost 73% of global consumption. While, the highest annual rates of growth in the last 10 years with regard to strawberry consumption were recorded in Mexico, Egypt and China.

Per capita consumption is also quite various, and in 2017 the highest consumption was recorded in Turkey (5.2 kg/year in 2016), followed by Egypt (4.9 kg/year), by U.S. (4.5 kg/year) and Europe (3.5 kg/year). In Europe, the first producer is Spain, followed by Poland.

Strawberry fruit initiates from a single inflorescence and is actually an aggregate, composed of many ovaries, each with a single ovule (Perkins-Veazie, 1995). The true fruit of strawberry are the seeds, called achenes, which are embedded in the epidermis of the swollen receptacle. The receptacle is composed of an internal pith, a cortex layer, and an epidermal layer (Suutarinen et al., 1998; Fait et al., 2008). Fibrovascular strands connect the achenes to the interior of the fruit, supplying nutrients to the achenes and the parenchyma cells. The achenes vary for position, size, shape and color, according to the cultivars. The mature achenes contain a relatively thick pericarp, a single layer endosperm, and a small embryo, whose formation is completed 10 days after anthesis (Nitsch, 1950). The false fruit, represented by the fleshy receptacle, follows mainly a single sigmoid curve pattern of development and growth. However, double sigmoid patterns have been reported for some cultivars.

In the first lag phase, auxin, synthesized in the achenes, promotes fruit growth, reaching a peak in concentration prior to the white stage, later declining as the fruit matures. The subsequent changes during strawberry maturation are accompanied by coordinated modifications in the levels of transcripts related to primary and secondary metabolism (Aharoni and O'Connell, 2002). Phytohormones greatly impact the faith of this development too: although the Rosaceae family, which includes strawberry, cherry and raspberry offers a multitude of fruit development patterns, common set of molecular and physiological events to achieve the final product can be found, not only for the sigmoid or double sigmoid patter, but also according to the hormonal content trends that can be drawn for the different fruit types (Rasori et al., 2010; Eccher et al., 2008; Farinati et al., 2017).

The major soluble sugars in strawberry are glucose, fructose and sucrose, which significantly increase during the development (Hancock, 1999), while minor soluble sugars, like inositol, xylol and galactose decrease (Moing et al., 2001). The main organic acids of strawberry are citrate, malate, and quinate, while minor organic acids includeacetate, oxalate, succinate, isocitrate, fumarate, and aconitate (Moing et al., 2001). Sugar and ratio between sugars and acids are major components of fruit quality dermination and consumer acceptance (Park et al., 2006). Amino acids are other soluble gear to fruit flavor, together with phenolic compounds. Phenolic compounds contribute to fruit color, flavour, and interact with mechanisms of pathogenic and UV protection (Aaby et al., 2005). A model for the cross talk between primary and secondary metabolism was proposed by Fait et al. (2008) with the aim of addressing the metabolic regulation underlying fruit seed development and the results suggest that changes in primary and secondary metabolism reflect organ and developmental specificities, highly coordinated during early development.

Nonetheless, a comprehensive view of the metabolic changes in the strawberry network during fruit development is still not completely defined and in particular for texture.

Some genes responsible of the metabolomics changes, mechanisms underlying the hormonal, transcriptomic and proteomic modifications occurring during fruit development, epigenetic mechanisms underlying heritable traits of agronomic importance have been studied, with particular regard to the exploitation of economically important phenotypes through breeding (Farinati et al., 2017). Mechanisms leading to the alteration of the structure of chromatin and changes in the subsequent gene expression, that can include DNA methylation, Post-Tranlational Modifications, which are highly organ specific, also in other crops (Bonghi et al., 2011), need to be further understood in their complexity but it is undeniable their contribution to the fruit quality determination.

Strawberry consumption is mainly driven by nutraceutical and convenience properties of strawberries. These last relate in particular to different urban habits and food “as it is”, making this berry the fifth highest consumed fresh fruit by weight, just after bananas, apples, oranges and grapes in the US (Grubinger, 2012).

With particular regard to Europe, strawberry demand has changed a lot in the last years and it has become a product, for which the consumer is willing to pay premium prices for a high quality product, low input produced, or organic, and often locally produced.

Future segmentation of quality-strawberry varieties on the market (Hancock and Simpson, 1995; Davik et al., 2000; Moser et al., 2011) is thus consumer oriented and assisted, starting from breeding programs.

Strawberry quality, although being a dynamic process that develops with the changes of the society, is fundamentally defined as an appealing, tasty and flavored fruit, better if without damages or decay signs (Montero et al., 1996; Casierra-Posada et al., 2011). Appealing fruit can regard mainly size, shape and color, which are quite measurable traits in strawberry. Tasty and flavoured fruits fall into more complex categories to be defined, both by consumers and scientists.

Strawberry texture is determined by the turgor pressure along with the unique arrangement and composition of the middle lamella and plant cell walls (Paduret et al., 2016).

Taste and aroma highly depend in strawberry on texture, volatile compounds, sugars, acids and phenolics. All of them are highly variable in the different phases of ripening, post-harvest mainly due to cell wall disassembly and loss of cell adhesion in the different tissues, The main enzymes involved are polygalacturonase, endoglucanase, β-galactosidase, β-xylosidase. This causes a general progressive change in turgor pressure and variation in flavor, due to changes of all the components involved (Figueroa et al., 2010).

Consumers appreciate strawberries when they are full ripe. However, being non-climacteric fruits, although they fully color after picking, they do not complete the ripening process off-plant and this can be problematic if the fruit is detached at the white or pink stages.

Fruit quality is also tightly connected with its stability during the entire pipeline: traits that facilitate strawberry handling like firmness, and for traits linked to grades and standards. As much as they are positive as much they would benefit processing operations.

Storage of fresh strawberries is very complex due to a high-metabolic activity, high rate of respiration (50–100 mL of C02 per kg per hour at 20 C) (Almenar et al., 2007). The short shelf-life can limit the marketability of this product, and losses can reach up to 40% during storage (Caner et al., 2008). Temperature in post-harvest has also a direct impact in fruit firmness (Kader, 1991; Perkins-Veazie, 1995). Textural changes in strawberry take place very fast: their high metabolic rates allow a very rapid fruit softening and subsequent fruit decay, causing tremendous losses during postharvest storage (Giongo et al., 2010).

Fruit quality and consumer liking suggest that genetic improvement of texture and fruit quality texture-related traits is a priority and is challenging. However, several fruit quality traits have been reported as being in linkage drag with other positive ones: sugar content, acidity, and total phenolic content were negatively correlated with fruit size (Capocasa et al., 2008) as well as fruit aroma and fruit firmness (Ulrich et al., 2014).

Quality driven plant breeding programs, need thus to rely on multiple, multifactorial and multi temporal determinations, considering all the most critical food-chain steps. In this view, the goal of this study was to dissect the texture triat of strawberry during fruit development on three cultivars, at harvest and postharvest on a set of 87 strawberry accessions.

MATERIAL AND METHODS

Plant material and sampling

Strawberry plants of the different cultivars were grown in soilless conditions in a typical mountain environment (520 meters above sea level), under high tunnel culture. The strawberry plantlets, all being certified, were transplanted in buckets (48x22x11 cm) with a peat volume of about 12 l, placing four plants in each bucket. An automatic fertigation system was used to guarantee water and nutrients supply while water conductivity was periodically monitored at 1300 µS with a conductivimeter (Crison Instrument Mod. CM35). With regard to the fruit development and phenological stage for sampling, the BBCH scale (Meier, 1994) was used, adapted to this study as described in table 4.1, and stage specific evaluations were conducted on the cultivars Candonga, Elsanta and Darselect.

Table 4.1 Phaenological stage used in sampling, according to the BBCH scale.

stage in this study	BBCH reference (Meier, 1994)	Fruit characteristics
0	71	10% of fruit formed, Receptacle protruding from sepal whorl
1	73	30% of fruit formed, Seeds clearly visible on receptacle tissue
2	75	50% of fruit formed
3	77	70% of fruit formed
4	79	Majority of fruit formed
5	81	Beginning of ripening: most fruits white in colour
6	85	First fruits start to red color (less than 30%)
7	87	Colouring advanced of the first fruits - red color on about 50% of the fruits
8	88	Colouring advanced of the first fruits – red color on more than 50% of the fruits
9	89	Majority of fruits harvested on the plant and fruit completely ripe.

At each stage, a minimum of 20 fruits were manually collected. They were as much homogenous as possible for size and color and free from damages, divided in two batches, one immediately nitrogen frozen for subtractive hybridization and sequencing, the other put in ice pack and processed for confirmatory analysis of the phaenological stage. Fruit quality attributes at each of the ten developmental stages were monitored using a chromameter (model CR300 Konica Minolta) calibrated to a white plate using CIE L*, a*, b* color space, where L* indicates brightness, a* axis from green to red and b*, from blue to yellow. Diameter (mm), height (mm), and single berry weight (g) were measured using a digital non-destructive compression fruit tester FirmTech2 (UP GmbH, Germany).

At the different fruit developmental stages, samples were determined also for total phenols content by the use of the Folin-Ciocalteau assay, total antioxidant activity through FRAP as described in Nicoletto et al., 2018.

Ninety-nine strawberry cultivars of the FEM collection were used for this whole study. Evaluations have taken place over the 2015-16 and 2017 years; there were cultivars that were not present for one year or vice versa. Nevertheless, a wide-ranging database for strawberry-quality attributes was collected. Strawberries were manually harvested according to their type, the June-bearing types were harvested in the summer of 2015 and 2016, this kind of strawberry produces a big crop load per year, typically around June. Everbearing strawberries, on the other hand, were harvested during both summer and autumn of the mentioned years, because this type of strawberries can produce during the entire growing season. Around thirty fruits for each genotype, free from external damages or irregularities, were sorted based on size and color homogeneity into two sub-groups, respectively, at harvest and after eight days of storage at (2°C and 95 % of RH) and analyzed.

Texture Analysis

Strawberry fruit texture was assessed by employing a texture analyser (Zwick Roell, Italy), using a destructive penetration test and a non-destructive compression test. The analysis profiled a mechanical force displacement using a 5 kg loading cell and a cylindrical flat head probe with a diameter of 4 mm, entering into the strawberry flesh transversally. The test was completed by penetrating the sample at a test speed of 300 mm/min and to a 99% deformation (strain).

The penetration test was preliminarily applied on three cultivars, Darselect, Candonga and Elsanta at four different developmental stages to set the main parameters, which were designed based on the force displacement profile. Initially, 14 mechanical texture parameters were used, in order to cover most of the trait variability. Filtering to avoid redundancy and increase efficiency of the dissecting technique and was then applied and seven parameters were chosen as follows: maximum force (FMax, N), minimum force (Fmin, N), final force (Ffin, N), area (Area, N mm), gradient parameters indicating the Young's modulus (G_YM, MPa), deformation at maximum and minimum force (Def_Fmax and Def_Fmin both expressed in %).

The texture profiling through penetration was carried out on 87 cultivars, assessed at harvest and after eight days of storage at 2°C and 95 % of RH.

Statistical Analysis

The data acquisition carried out on the mechanical profiles was operated by the software Exponent v.4 (Stable MicroSystem, Ltd., Godalming, UK) provided with the Zwick instrument. With the same software a macro instruction was also compiled to automate the parameter extraction from the profile to excel. Data analysis was performed with Statistica 13 software for the multivariate analysis Principal Component Analysis (PCA) and ANOVA.

RESULTS AND DISCUSSION

Quality characteristics during fruit development

During fruit development, pomological and colorimetric characteristics changed for the three cultivars analysed according to the stage. In Elsanta, similarly to Darselect, as resumed in table 4.2, lightness increased from stage 0 to 5, decreasing then rapidly until overripe stage. Chroma Index, inferred by the chromatic values a* and b*, shows an increase in values until stage 5, reaching then a plateau until stage 8, decreasing rapidly afterwards. Single berry weight, as well as fruit diameter and fruit height increase until stage 4, not changing significantly until stage 7 and increasing then again, according to a double sigmoid pattern, as described by Rasori et al., 2010; Eccher et al., 2008; Farinati et al., 2017. The ripening process in strawberry is relatively rapid and typically occurs 5 to 10 d following the white stage.

When the pomological characteristics of Elsanta are analysed by principal component analysis (Fig. 4.1), the ten stages are clearly distributed over the four quadrants.

Table 4.2: Pomological characteristics of Elsanta strawberry at the relative stage of fruit development for color space (L*a*b) indicating respectively lilghtness, a* is the red/green coordinate, and b* is the yellow/blue coordinate. SBW represents the single berry weight expressed in grams. Diameter and height of the single fruit are expressed in mm. For each parameter standard deviations are given.

Stage		L*			a			b			SBW (g)			Fruit Diameter (mm)			Fruit Height (mm)	
0	43,79	±	4,72	-3,06	±	1,49	39,58	±	7,93	0,64	±	0,22	10,49	±	1,4	12,6	±	1,73
1	56,23	±	2,16	-3,71	±	0,75	33,48	±	1,63	2,61	±	0,84	19,3	±	1,95	21,61	±	3,36
2	57,09	±	1,72	-2,77	±	1,13	33,92	±	1,41	6,42	±	1,08	24,42	±	1,86	28,76	±	2,04
3	55,94	±	2,01	-3,73	±	0,51	33,18	±	2,42	9,84	±	1,94	27,06	±	1,84	28,57	±	7,34
4	57	±	3,18	-2,13	±	0,8	32,72	±	3,31	14,91	±	3,36	26,24	±	4,63	34,63	±	3,47
5	58,79	±	3,07	-1,3	±	1,61	28,98	±	3,12	11,51	±	2,63	30,03	±	3,14	29,2	±	3,59
6	50,98	±	8,27	12,36	±	13,23	26,61	±	2,89	12,13	±	1,89	30,49	±	2,22	32,62	±	9,13
7	49,64	±	11,3	15,82	±	14,06	23,89	±	2,68	12,47	±	4,04	29,43	±	4,96	27,5	±	5,48
8	35,39	±	2,79	27,87	±	3,76	19,64	±	4,19	15,76	±	4,45	32,33	±	3,45	28,64	±	4,81
9	30,67	±	3,18	31,95	±	5,01	20,75	±	6,61	21,43	±	11,03	33,67	±	5,8	29,2	±	8,77

When only the cultivar Elsanta is analysed over the 10 stages by the use of PCA , a clear dissected pattern for morphological parameters can be seen. The PCA (Fig. 4.1) explains a high variation (93 %) when the morphological parameters are used to describe the fruit at different ripening stages, mainly according to PC1 that explains more than 70% of the variation and is referred to both SBW and a* values. The changes during strawberry maturation are accompanied by coordinated modifications in the levels of transcripts related to primary and secondary metabolism (Aharoni and O'Connell, 2002) and in the first developmental stage, the length of cell division plays a relevant role in determining the final fruit density and size at harvest, and several fruit quality traits are strongly linked to this parameter (Farinati et al., 2017).

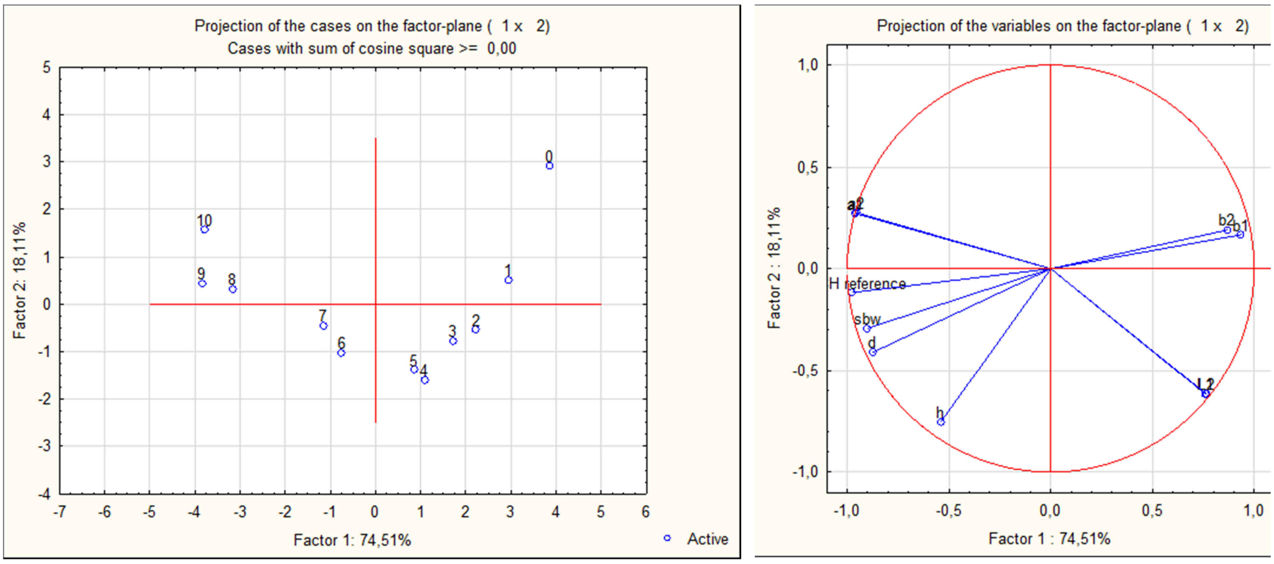

Figure 4.1 PCA representing the nine stages and stage 10 (overripe fruit) relating to color, size and single berry weight characteristics of the fruit at the corresponding stage and respective loadings.

Analyzing fruit texture, in the first four developmental stages examined – from small green to fully expanded white fruit – the internal fruit structure has clearly a lower impact on the texture shape. An example of strawberry texture profile is presented in Fig 4.2. Three distinguishable force peaks are evidenced, which indicate the opposing resistance forces of the fruit when the probe penetrates the fruit anatomical structures. First, the breaking of the hypodermis and cortical cells until reaching the internal pith; second, the pass through the pith cavity and finally, the trespassing of the other layer of cortical cells and vascular bundles connected to the other side of the fruit (Giongo et al., 2010). Being the second and the third peaks highly influenced by several compacting tissues that oppose a cumulative force to the probe, only the first peak was considered as resolutive for maximal force indication.

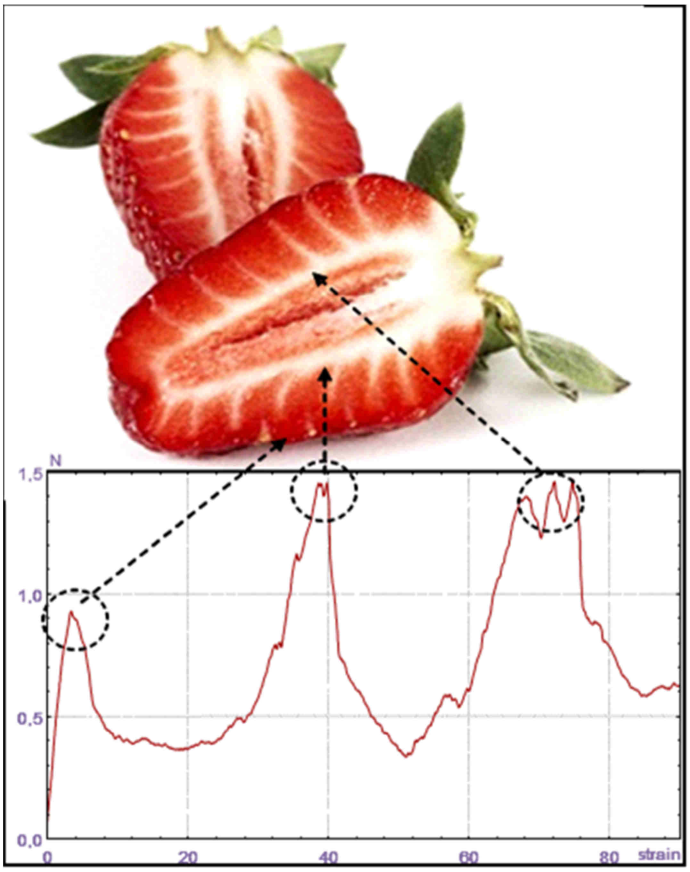

Figure 4.2 Example of strawberry texture profile: Force (N) in the y-axis and strain (%) in x-axis.

Strength profiles show a decrease in the cultivar Darselect from about 50 N in stage 1, to 26 N in stage 4. Also the profiles of Elsanta showed a similar shape of the force displacement profile. From stage 5 (large green) to stage 10 (fully ripe), the shape profile resulted in a decrease of force, from twenty to five-fold less compared to the initial stages.

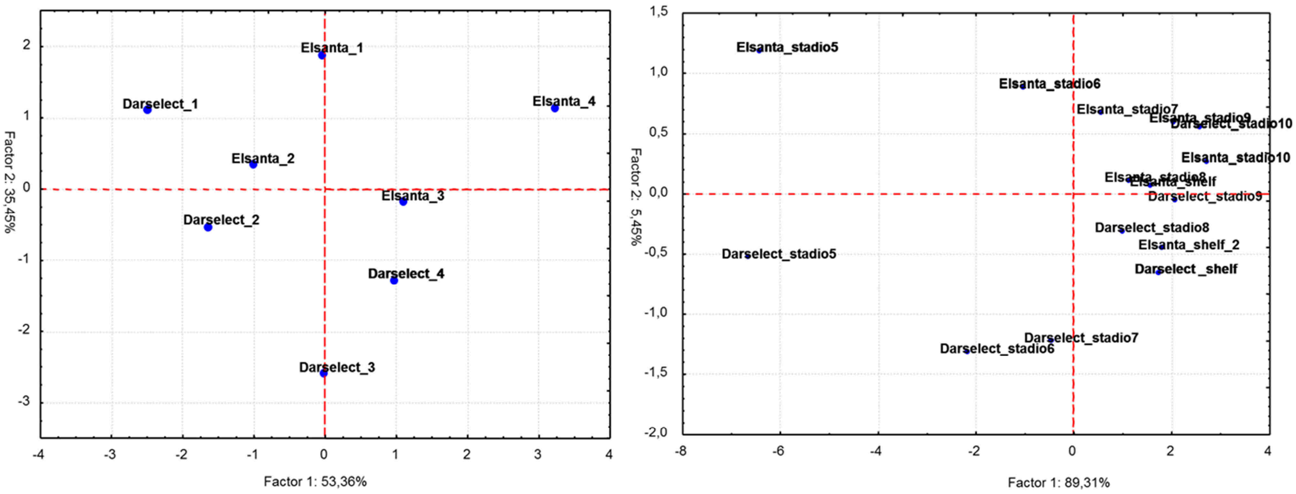

Figure 4.3 Development texture profiles from stage 1 to 4 in the first PCA and 5 to 10 in the second for the cultivars Elsanta and Darselect.

Stage 5 might be pointed out as the most critical for the change in complexity of the ripening process in terms of hormonal control, enzymatic modifications and oxidative stress involved in general ripening program of this non-climateric fruit (Aharoni et al., 2002; Suutarinen et al., 1998). For all 5-10 stages the profiles highlighted three distinct peaks evidencing the internal fruit structure, clearly defining the vascular bundles, the internal cylinder calyx and pith of the receptacle and again the second layer of vascular system components connected to the achenes.

Darselect and Elsanta were analysed for their developmental profile during three production seasons: summer, late summer and beginning of autumn. Here we report the results for June. In fig. 3, Darselect and Elsanta, cultivars with similar agronomical properties, were distinguished in the PCA plot regarding the 1-4 stages profile. Darselect shows higher values compared to Elsanta, being the stage 2 less discriminating.

A clear determination was also reported in the stages from 5 (large green fruit) to 7 (expanded fruit red color≤50%), with Elsanta and Darselect clustered apart. Close to mature stage they tend to group together, keeping however a slight difference in textural performance. At fully mature stage they overlap, showing similar ripening properties.

Differences among ripening stages are significant also when the two cultivars are analysed for texture profiling and the PCA shown in Fig. 4.4 describes a high variation (96 %) dissected through five more resolutive penetration parameters used to describe the texture at different ripening stages. Although the genotypic values of the three cultivars at the single stage show variation, the pattern is the same for all of them, including the texture behaviour after 3 days of chilling, considered a shelf life index.

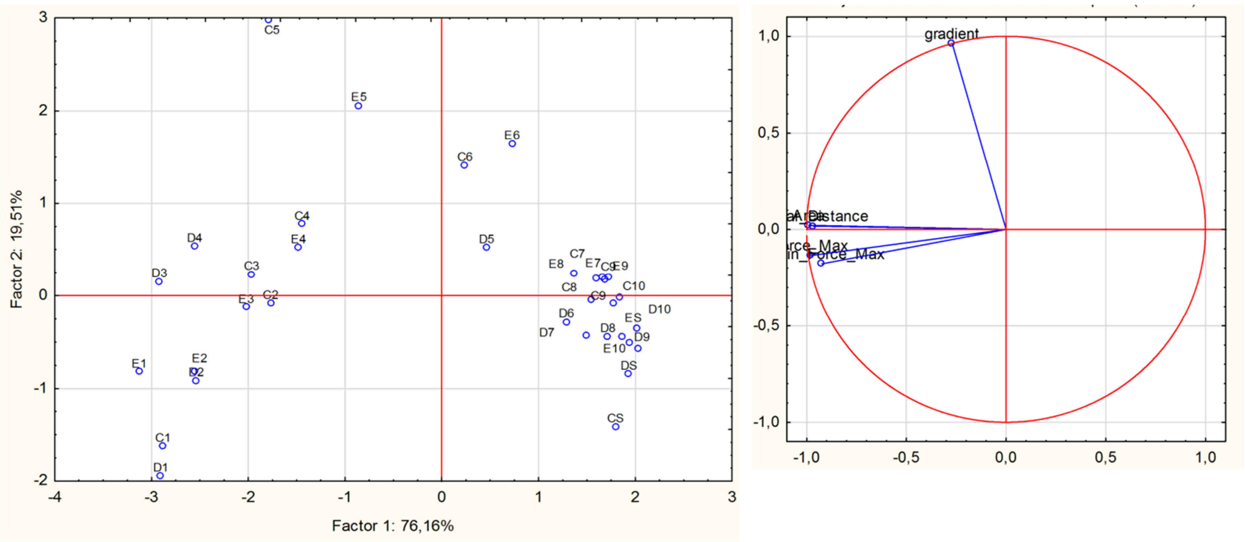

Figure 4.4 PCA representing the 10 stages and stage SL (shelf life fruit) relating to texture mechanical parameters of the fruit of Candonga, Elsanta and Darselect at the corresponding stage and respective loadings.

The direction of the loadings distinctly separates according to all the forces, and PC1 explains 76 % of the variability, grouping all the samples related to stages 0-4 in the second and third quadrants from the other stages in the two opposite quadrants. PC1 (76 %) is mostly defined by forces parameters, area of the forces and deformation, while PC2 (17 %) explains the variation based on the Young's module.

The levels of total polyphenols (TP), FRAP, vitamin C, quercetin -3-gal, delphinidin -3-glu, cyanidin -3-glu, catechin, epicatechin, chlorogenic acid, caffeic acid, coumaric and cinnamic acids were also quantified according to the relative fruit developmental stage for Darselect and Elsanta.

Also the PCA (Fig. 4.5) built on the secondary metabolites explains a high variation (82 %) when the different parameters and compounds are used to describe the fruit at different ripening stages. PC1 explains 69% of the variation and is referred mainly to the total polyphenols parameters, while the single compounds contribute to the PC2 explanation for about 14%.

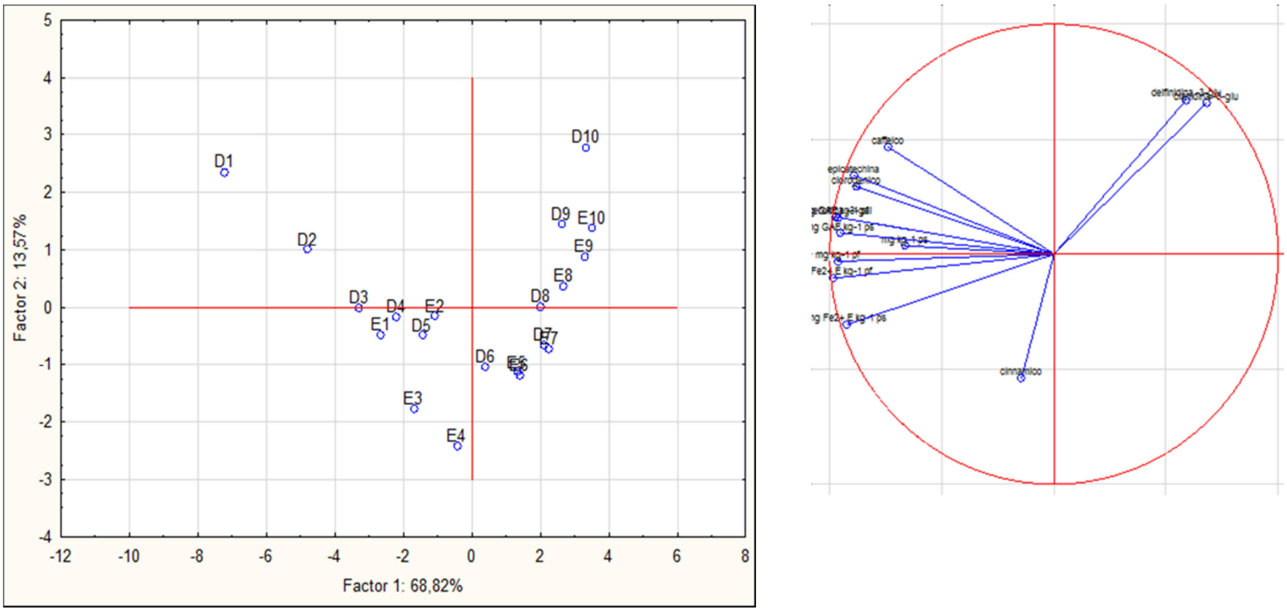

Figure 4.5 PCA representing the 10 stages and stage SL (shelf life fruit) relating to secundary metabolites of the fruit of Elsanta and Darselect at the corresponding stage and respective loadings.

Higher content of total polyphenols and individual compounds like vitamin C, quercetin -3-gal, catechin, chlorogenic acid, caffeic acid, coumaric and cinnamic acids are evident in the very first stages of development, which decrease until stage 10 in both cultivars. On the contrary, delphinidin -3-glu, cyanidin -3-glu increase their fruit concentration from stage 1 to stage 10 and Vitamin C maintains a stable concentration after stage 5 as well as epicatechin.

Phenolic compounds contribute to fruit color, flavour, and interact with mechanisms of pathogenic and UV protection (Aaby et al., 2005). The literature supports this resultswith flavanols, mainly tannins, accumulating during early stages, (Halbwirth et al., 2006) to high levels and providing immature fruit an astringent flavor (Aharoni et al., 2002). In later stages, other flavonoids, such as anthocyanins and flavonols show a second peak (Almeida et al., 2007; Landmann et al., 2007).

Texture profiling of the germplasm at harvest

A high genetic variation was explained (90%) among the 87 strawberry cultivars when texture was objectively analysed by the use of 7 texture parameters at harvest.

70% percent of the total texture variability was accounted for the first principal component and data vary in relation with changes of the area, maximum and final force loadings, thus related to the physical stress employed to rupture the fruit until the pith cavity. The second principal component, which determines changes in the vertical axis, is heavily influenced by deformation values and Young’s modulus, both parameters that concern the compression resistance and their fruit elasticity. They are independent from the other force parameters, being close or completely orthogonal in terms of direction of the respective vector direction.

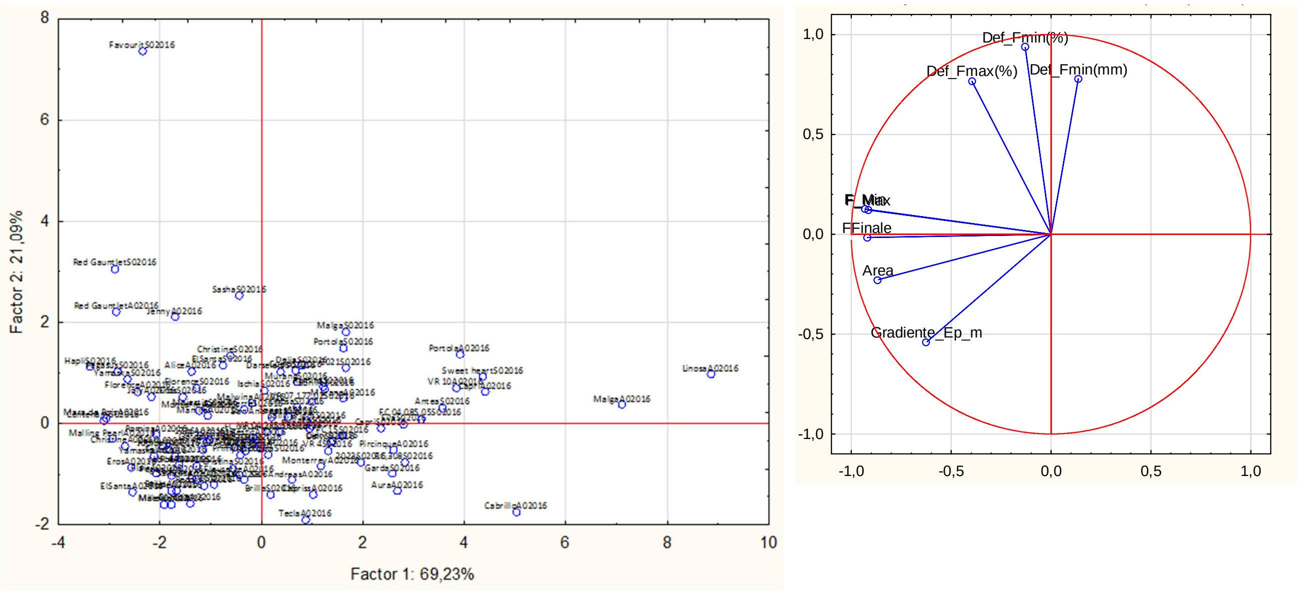

Figure 4.6 PCA representing the distribution of 87 cultivars at harvest dissected according to texture mechanical parameters and respective loadings.

The chosen parameters clearly distinguish the genotypes, identifying those that outperform for the different mechanical parameters. Favourit in the summer production, shows the highest value of deformation, while Linosa, Malga and Cabrillo in autumn growth show the highest cumulative values of internal forces, although this does not reflect that they are the most representative positive genotypes for the individual force parameters.

Different genetic background contributes to cultivars clustering according to their breeding program provenience. The PCA, related to texture components, gives a picture of various breeding programs objectives and separates, although not always clearly, varieties and advanced selections that derive from different distinguished sources.

In the autumn production the most important discriminating factor seems to be associated to day-length sensitivity. There is a major distinction between everbearing strawberries, both from Californian and European programs, and junebearing varieties, that perform negatively regarding texture components. Furthermore, junebearing varieties more suitable or bred for soilless management and for plain soil group apart each other.

Texture profiling of the germplasm after storage

The texture penetrometric parameters were used also to dissect the variability present among the 87 cultivars, representative of the strawberry genepool. The first two principal components of the PCA related to post-harvest distribution, based on the same 7 descriptors used in the penetrometric texture analysis at harvest, accounted for about 80 % of the total variation in the data. The 87 cultivars are more spread over the four PCA quadrants than the previous timepoint, indicating clear different textural patterns and high variability among them. The vectorial direction of the parameters is sable compared to harvest, confirming and validating the efficiency of these parameters in dissecting the texture trait also after metabolic process that occurred after ripening.

Again, PC1, which accounts for an explanation of about 50 % of the variability among cultivars, defines those that show mainly high values of F_Max, Area, F_Fin and F_Min, thus internal forces. The cultivars are mainly distributed for these parameters in the second and third quadrants. They are separated from those that show an opposite trend and are spread over the first and fourth quadrants. The loadings of Young's modulus are even more consistent with the situation observed at harvest, and this parameter is strongly orthogonally oriented with the fruit resulting deformation at maximum and minimum forces.

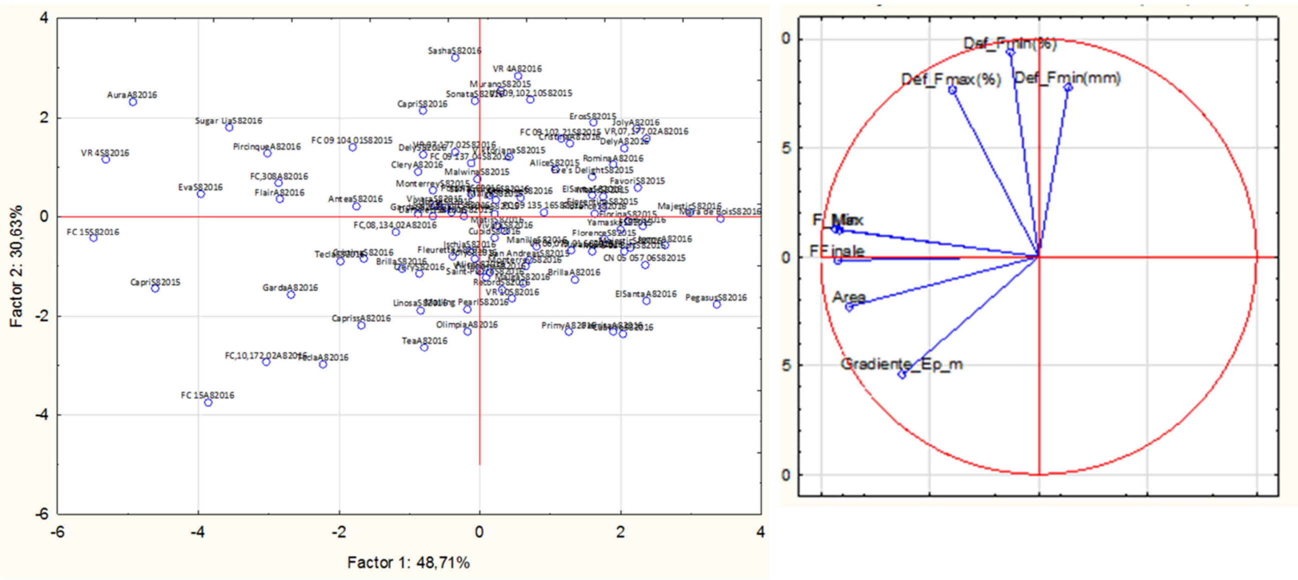

Figure 4.7 PCA representing the distribution of 87 cultivars after storage dissected according to texture mechanical parameters and respective loadings.

This vector independence denotes their value in dissecting the variance among cultivars. In addition, the two parameters can thus be interpreted as describing texture properties referred to external tissues of the fruit, namely elasticity and skin toughness. It is evident that they have a different impact on shelf life ability contributing to the outcome performance for the fresh market of the different varieties.

The vector distribution indicates positive texture attribute in post-harvest for the internal tissues of the selection FC15 for the elasticity component, while Sasha, Murano, Sonata and Capri for skin toughness. The cultivars that perform better in terms of cumulative internal forces are Aura, Capri, FC 155 and VR 458. In order to have a more exhaustive view of the fruit texture parameters behaviour of the different cultivars, a dissection according to the trend of each genotype from harvest to post-harvest was conducted. This has the advantage to furtherly segment the different varieties into groups that shows positive or negative attributes related to texture, for a particular texture parameter, that in the end is in relationship with the characteristics of specific tissue layers. This phenotypic information can be of value for breeding purposes.

In particular, if some varieties are stable for their low values of all the parameters, like Pegasus, Yamaska and Mara de Bois, or for positive values, like Capri, some of them are very divergent when texture mechanical parameters mirroring external or internal forces are chosen.

Similarly, for the different cultivar the variation of the texture parameters changes from harvest to post-harvest is highly different and among parameters it can have not always the same trend. Garda for example shows increasing values from the two timepoints for FMax, while both the Young's modulus and F_Fin decrease during post-harvest. Young's modulus, in general presented a considerable variability among cultivars. A general decrease of Young's modulus was observed after storage. The lower the value of Young's modulus the more flexible or elastic a fruit is, contrarily, fruits with higher values can be considered stiffer or firmer. However, even if more elastic, in this case, the force peak increases from the inner tissues, compensating in a view of handling or touching the fruit.

For strawberries, freshness, firmness, taste, fruit color, and fruit size as the important factors for consumer decisions to purchase strawberries (Safley et al., 1999) and less willing to buy strawberries with low sugars content (Keutgen and Pawelzik, 2007). Also more recently, studies found consumers preferred a firmer strawberry cultivar with complex flavors (Lado et al., 2010; Colquhoun et al., 2012). Wang et al. (2016) identified three categories of strawberry consumers “Balanced Consumers,” “Experience Attribute Sensitive Consumers,” and “Search Attribute Sensitive Consumers.” that can be identified as target markets, but also help breeding to address them. A recent study indicated that not only consumers but also producers preferred to grow firm strawberries with intense flavor, ideal external and internal red color, and longer shelf life (Choi et al. 2017).

A study reported the genetic control of 17 agronomical and fruit quality traits in a F1 strawberry population over three successive years Zorrilla-Fontanesi et al., 2011). Together with plants characteristics and yield, fruit firmness using a penetrometer, soluble solids content and fruit color were measured for QTL analysis. 33 significant associations with QTLs were found, and a QTL for fruit firmness was confirmed, that was previously located in the same chromosomic region as Fa-Exp2, an expansin specifically expressed in strawberry fruits (Dotto et al. 2006). A more consistent phenotypic approach applied to genetic association might also be useful in order to identify more precisely QTLs for texture, thus advancing towards a molecular assited selection (MAS) approach.

The clear dissection of texture in parameters that can define a perception but also refer to different morphological structure of the fruit can give additional information to be used as biomarker in the selection process through breeding in a more objective way to find new products to make available to consumers.

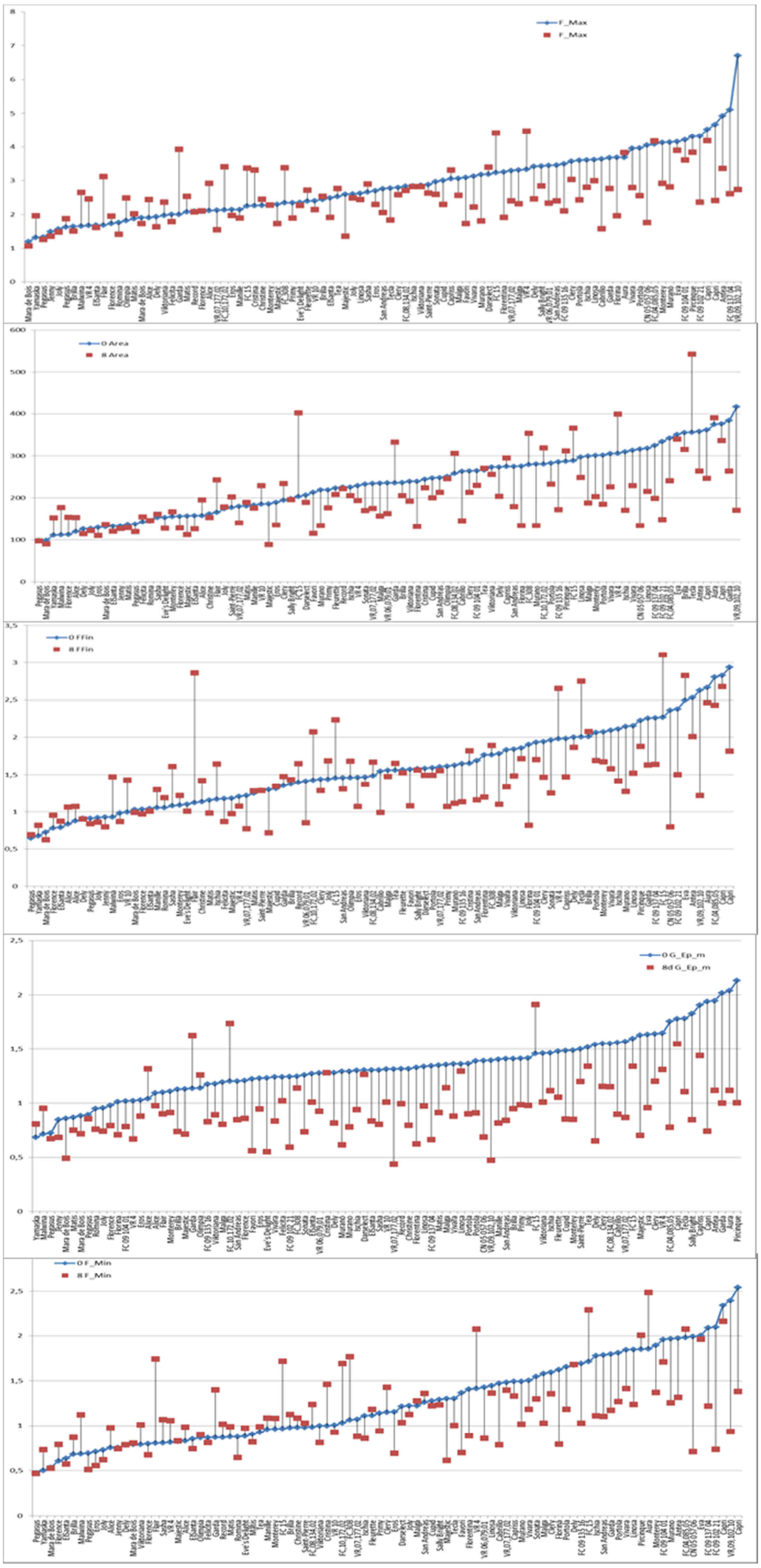

Figure 4.8 Bar plot F_Max, FFin, Area, Young's modulus, F_min, indicating changes between harvest and storage of the germplasm evaluation recorded. The segment that connects both colors is the difference between the two values. Numbers on the x-axis represents the code of the strawberry cultivars.

CONCLUSIONS

Strawberry fruit quality is now an imperative demand by consumers, growers and intermediaries and it is strongly correlated with better texture. This implies texture to be known and measured to tailor breeding programs according to it.

The present study includes a broad germplasm assessment as well as different limiting timepoints of the industry chain: harvest and post-harvest. 87 cultivars were characterized for both parameters at harvest and after eight days at 4°C.

A modern approach, much more complex than a single point penetrometer measure, such as the texture analysis, described herein, is a suitable tool to assess thoroughly the complexity of this important quality attribute of strawberry during diverse storage conditions. The effect of storage time was clearly reflected in the textural profile and variable in behaviour according the cultivar.

Enhancement of strawberry fruit quality for texture traits seems to be more feasible, once that, according to different mechanical parameters used, the texture attitude of a cultivar is dissected and possibly be used as a donor parent.

As far as softening proceeds, the degree of alteration of the strawberry quality is strictly dependent on the cultivar. Thus, the evaluation of texture in post-harvest can help not only growers for cultivar choice, but also to direct storage and sorting to different storage attitude of the cultivars.

The results allow also for an additional segmentation of the strawberry fruit, according to texture characteristics objectively measured.

Chapter V

Fruit quality of sweet cherry (*Prunus avium* L.): harvest, postharvest and rootstock effects on cultivar texture profiling

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GL conceived and designed the research, coordinated the experiment, analysed the data and wrote the chapter. All the authors contributed to the research. MF contributed to texture analysis and morphological data. FC made available the texture analyzer. BF contributed to data evaluation. PS revised the chapter. GB made all the samples available and discussed the results before writing. CB revised the chapter and contributed to the interpretation of the results.

INTRODUCTION

Sweet cherry (*Prunus avium* L.) is a popular temperate fruit, mainly because it is early and fruit quality is highly appreciated by consumers. The world production is about 2,200 Mtons in 2015 (FAO source), the main producers being Turkey (417 Mt), United States of America (287,305 t), Iran (255,500 t) and Italy (115,476 t), with a yield of about 40 q/ha on a total surface of about 28.000 hectares. The harvest window and storability of the cherry varieties is quite limited although they are key factor to commercialization. Furthermore, while technologies to preserve the physical and chemical properties of cherry fruits have widely been reported (Monzon et al. 2006; Marquenie et al. 2002; Romanazzi et al. 2008; Martinez-Esplà et al., 2014) and adapted to the production and commercialization pipeline, less has been done in the last decades to characterize the germplasm or improving specific traits through breeding. Because of this, the need of novel accessions with a more extended ripening time and better shelf life is a priority for both growers and retailers.

In general, the concept of fruit quality in cherry includes sensory attributes, nutritional values, chemical compounds and physical properties. For this latter category, the most important features are represented by fruit texture, size and colour. Among them, the modification of the fruit texture occurring during the fruit ripening and postharvest play a pivotal role in the definition of the general fruit quality as well as the economic success of a cultivar. It is in fact this property that impacts for the most the consumer appreciation and the maintenance of the fruit quality during storage. The importance of the fruit texture study is documented by recent publication of several studies focused on the dissection of the fruit texture in different species, such as apple (Costa et al., 2012), blueberry (Giongo et al., 2013), and table grape (Rolle et al., 2011). In these studies, the need of more tuned instruments has also been emphasized in order to unravel at high definition the several texture components, being this a complex trait composed by multiple components.

The aim of this study was the evaluation of a set of fruit quality traits, with particular regard to fruit texture, of a wide set of commercial sweet cherry (*P. avium* L.) cultivars grown worldwide. Furthermore, a storage index was defined, in order to profile a postharvest behaviour for cherry.

MATERIALS AND METHODS

Plant materials

In this study a total number of 36 cultivars of sweet cherry (Table 5.1) were chosen from an experimental field of Istituto Sperimentale per la Frutticoltura (Provincia di Verona), in the north of Italy. At the time of the analysis, plants were in the full production phase, between 12 and 15 years old represented by three plants each cultivar. Trees were grown on different rootstocks as described in Table 5.3, with a plant density of 5 x 3 m. Water supply was guarantee by an irrigation system, and the trees were maintained following standard agronomical practice for pruning and disease chemical control.

Table 5.1 List of cultivars of the different experiments and settings.

CV	Other registered name cultivar/Selection	Presumed parentage	Origin	Self (in)compatibility status
Black Star	Black Star	Lapins · Burlat	BO, Italy	SF
Blaze Star	Blaze Star	Lapins · Durone compatto di Vignola	BO, Italy	SF
Brooks	Brooks	Rainer x Early Burlat	BP, USA	
Carmen	Carmen	Yellow Dragan x H203	Hungary	
Coralise	Coralise (Gardel)	Unknown		
Critalina	Cristalina	Star x Van	BC,Canada	S1S3
Danelia	Daneliya	Hedelfinger x Germersdorfer	Kyustendil - Bulgaria	
Duroncino Costasavina	Unpatented	Unknown	Italy (Trentino a/Adige)	

Enrica	Enrica	Vittoria x C2.27.12	Italy (ISF-Verona)	SF
Ferrovia	Unpatented	Unknown	U, I	S3S12
Giorgia	Unpatented	Sel. ISF 123 x Caccianese	Italy (ISF-Verona)	
Grace Star	Grace Star	Burlat lib. imp.	BO, Italy	
Hartland	Hartland	Windsor lib. Imp	Geneva USA	
I 69	Unpatented		Italy (ISF-Verona)	
Index	Index TM	Stella x unknown	Wa, USA	self fertile
Kordia	Libera da brevetto	Libera impollinazione di Semenzale di Random	Repubblica Ceca	
Lalla Star	LaLa Star	Compact Lambert x Lapins	Italy	S1S3
Lapins	Lapins	Van×Stella	BC,Canada	S1S4[1]
Late Lory	Unpatented	Hedelfinger x Tardivo di Vignola	France	
Linda	Unpatented	Hedelfingeni Orias x Germersdorfi Orias		
Lucrezia	Unpatented	Vittoria x C.2.22.12	Italy (ISF-Verona)	
Mora di Verona	Unpatented	Unknown	Italy	
Nero II	Unpatented	Unknown	Italy	
New Star	New Star	Van x Stella	Canada	
Prime Giant	Prime Giant	Large Red x Ruby	USA	self infertile
Regina	Regina	Schneiders Spate Knorpelkirsche x Rube	Germany	S1S3
Sandra Rose	Sandra Rose	2C-61-18×Sunburst	BC,Canada	S3S4[1]
Santina	Santina	Stella×Summit	BC,Canada	S1S4[1]
Somerset	Somerset	Van x Vic	NY, USA	SF
Sweet Earth	Sweetheart® Sumtare	Van×Newstar	BC,Canada	S3S4[1]
Sylvia	Unpatented	Van x Sam	BC,Canada	self infertile
Symphony	Symphony	Lapins x Bing	BC,Canada	self fertile
Tieton	PC 71446	Stella x Early Burlat	WSU - USA	S3S9
Van	Van	Empress Eugenie op	Summerland,Canada	S1S3
Vigred		Germersdorfer' x Burlat	Slovenia	
VR 1255	Unknown	unknown	unknown	unknown

Fruit quality control assessment

Cherry fruits were randomly selected, manually harvested and placed in ice packs for overnight storage in a refrigerating cell (3-4°C). The maturity stage was considered between S12 and S13 according to the maturity stage indicated by Serrano et al. (2005). For postharvest measurements, a second batch of cherries was stored for 14 days at 4°C with a relative humidity of 85%. Prior to instrumental analysis, stored fruits were kept for 1 hour at room temperature (~20°C), and ten fruits per cvs were used for analyses.

In addition to the texture parameters identified, other fruit quality parameters were measured, employing an additional set of 20 berries for each variety. Fruit diameter (mm) and fruit mass (g), soluble solid and acidity content were measured with a DBR35 refractometer and a titration Compact Titrator (Crison, Modena, Italy) and with 0.1 N NaOH of 5 g of berry, respectively and data were expressed in °Brix and meq/100g of fresh weight. Colour measures were conducted by spectrophotometric means through a Minolta device for L, a*, b* parameters. Fruit firmness was measured using a digital fruit firmness tester (TR Turoni srl, Forlì, Italy) with a 20 mm flat compression probe held on a stand on 10 fruit samples for each cultivar for three replicates.

Fruit texture evaluation

Cherry fruit texture was measured by employing a TAXT*plus* texture analyzer (Stable MicroSystem Ltd; Godalimng, UK), which profiled a mechanical force displacement using a 5 kg loading cell and a cylindrical flat head probe with a diameter of 4 mm entering into the cherry flesh from the sagittal side. The mechanical profile graph was based on two fundamental variables: force (N) and distance (strain, %). The force was measured with the following instrumental settings: test speed of 100 mm/min, post-test speed of 500 mm/min, auto force trigger of 5 g and stop plot at target position. Each single fruit was compressed until deformation of 13 mm. From the mechanical profiles, digital data were acquired with a resolution of 500 pps (points per second). On the force displacement profile, four parameters were specifically isolated, in particular: maximum force, gradient (or Young's module, also known as elasticity module), force linear distance and area, as indicated in table 5.2.

Table 5.2 List of mechanical parameters related to the texture cherry profiling.

Mechanical parameters	**General description**	**Unit**
Max force	Maximum force value recorded over the probe's travel, indicated here as Force 1 (as FMax in other studes).	N
Area	Area underlying the mechanical profile	N%
Force linear distance	Computation of the forcecurve length, indicated here also as Distance.	–
Gradient	Young's modulus or elasticity modulus, computed as ratio between stress and strain.	N%

A storage index (SI) was computed using the formula SI= $\log_2$(T*i*2M/T*i*H), with ΣT*i*H is the sum of the values of the '*i*' texture parameters measured at harvest, and T*i*2M is the value of the same parameter measured after two weeks of cold storage. Positive SI value indicate a texture sub-trait enhancement, whilst negative values point to a loss of textural performance during storage. An SI equal to zero means that between harvest and postharvest were not detected any difference.

Year and rootstock effect

The effect on texture at fruit maturity over two harvesting seasons was assessed as described above. Furthermore, texture analysis of five distinct sweet cherry rootstocks ('GiSelA 5', 'GiSelA 6', 'Piku 1', 'PHL-C', Colt and 'Weiroot 158') on the fruit texture of two scion cultivars ('Regina' and

‘Kordia’) was evaluated. While rootstocks Piku 1, GiSelA 5, GiSelA 6, Weiroot 158 and P-HL-C were common to both varieties, GiSelA 3, Weiroot 72 were tested exclusively on Regina and Colt on Kordia. All measurements were taken on randomly selected fresh sweet cherry fruits at ripening stage. Plants were located in the same experimental site in Verona, with the same planting density, management and sampling described above for cultivars comparison.

Sensory panel test

Acceptability assessment by ten untrained panelists was tested for texture characteristics and perception after tasting of 9 sweet cherries cultivars. They were asked to describe and evaluate a set of 9 sweet cherries simultaneously for three main triats according to a scale 1-10 scoring. They were asked to score ‘Aroma perception liking’, ‘Texture acceptance’, ‘Pulp liking’, ‘Skin liking’ and ‘General liking’. These descriptive scores where then correlated with texture mechanical parameters measured on the same bunch of fruit, the same days of the panels. Linear regression analysis for the different attributes was calculated, as well as Principal Component Analysis.

Data analysis

The data acquisition carried out on the mechanical profiles was operated by the software Exponent v.4 (Stable MicroSystem, Ltd., Godalming, UK) provided with the TA-XT*plus* instrument. With the same software a macro instruction was also compiled to automate the parameter extraction from the profile. Basic *post hoc* descriptive statistical analysis LSD-ANOVA, Pearson’s correlation analysis, and multivariate statistics (Principal Component Analysis, PCA), were carried out using the Statistica 13 software.

RESULTS AND DISCUSSION

Quality and texture cultivars comparison at harvest

Among the 36 cultivars that were evaluated data shown a significant variability among samples relating both to morphological traits and texture parameters. The morphological parameters can be compared with previous studies for the most known varieties, while some other have never been reported before for firmness and fruit quality, like Duroncino di Costasavina, Durone Nero II, Durone Nero III, Mora di Verona and the two selections VR 1255 and I62. The three main quality physicochemical characteristics considered were fruit mass, color and texture, as in several publications they are underlined as key choice factors (Sloulin, 1990; Muskovics et al., 2006; Estia et al., 2002; Bernalte et al., 1999; Toivonen et al., 2004). They all varied significantly ($p<0.00001$). Fruit weight, which is known to be under the effect of several environmental factors, was significantly higher in Linda (13.2 g), followed by Carmen (13.2 g) and Regina (11 g) and lower in Duroncino di Costasavina (6.1 g). Fruit size and weight are key traits for commercial market (Abas Wanib) and large fruit are generally preferred by consumers for visual appeal (Blazkova et al., 2002). Fruit size is in general negatively correlated with firmness (Sam et al., 1999), although in this study this showed variability among cultivars.

Relating to color at maturity (Fig. 5.1), the germplasm displayed a wide variation, which is well explained in the Chroma Index value that was between a maximum value of 21.7 of the cv Tieton and a minimum of 8.1 of the cv Black Star. Color represents one of the key indicators of maturity and quality of cherry and it is highly correlated (Esti 2002, Wang 97) with anthocyanins content, thus antioxidant potential. Consumption patter are highly driven by health awareness and cherry, which is one of the 20 richest fruit can play an important role in disease prevention and contribution to an healthy diet. The variation of the L* component (Fig. 5.1), which shows the lightness of the sample surface thus again involved in visual appeal of the fruit, is more variable in this genepool: a cluster of cultivars of high values of L*, lighter, is represented by Symphony, Late Lory, Lalla Star and Sweet Earth, while Badacsoni, Cristalina, Gegè, Grace Star, Index and Lapins, show lower values, indicating less light fruits.

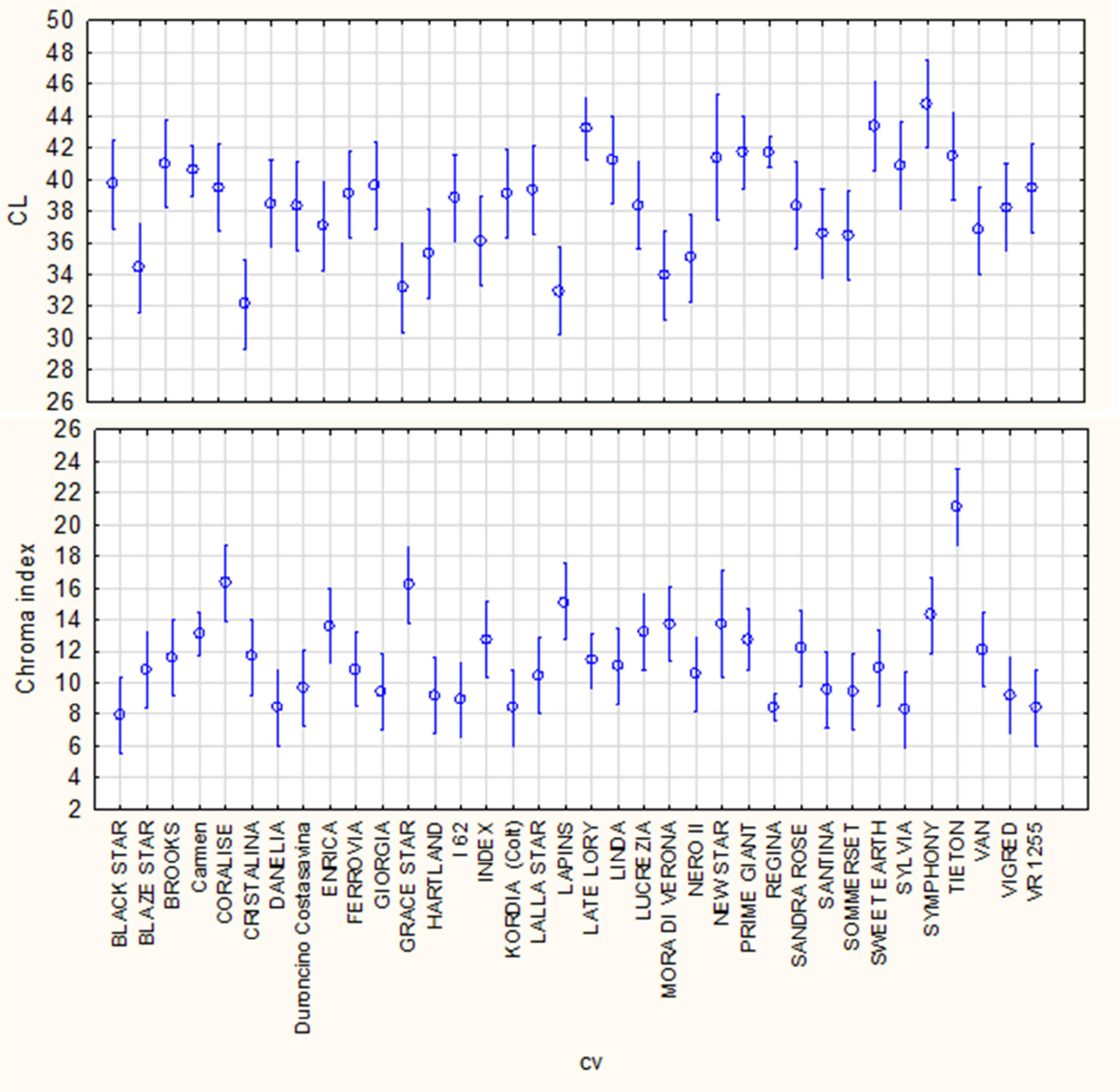

Figure 5.1. Results of the ANOVA analysis for the chroma values L* and Chroma index. Bars indicate interval confidence of 0,95. p=0,0001.

Firmness measured by the use of the penetrometer differed significantly in particular for Duroncino di Costasavina and Tieton for high values (2,03 N and 2,01 N respectively), while soft attitudes were measured on Danelia, which was the lowest (0,65 N), followed by Grace Star (0, 66

N). It is interesting to notice that 34 cultivars out of 36 were below the threshold of firmness of 1,5 N, indicating a strongly screwed distribution of this trait when it is measured by single point means (Fig. 5.2).

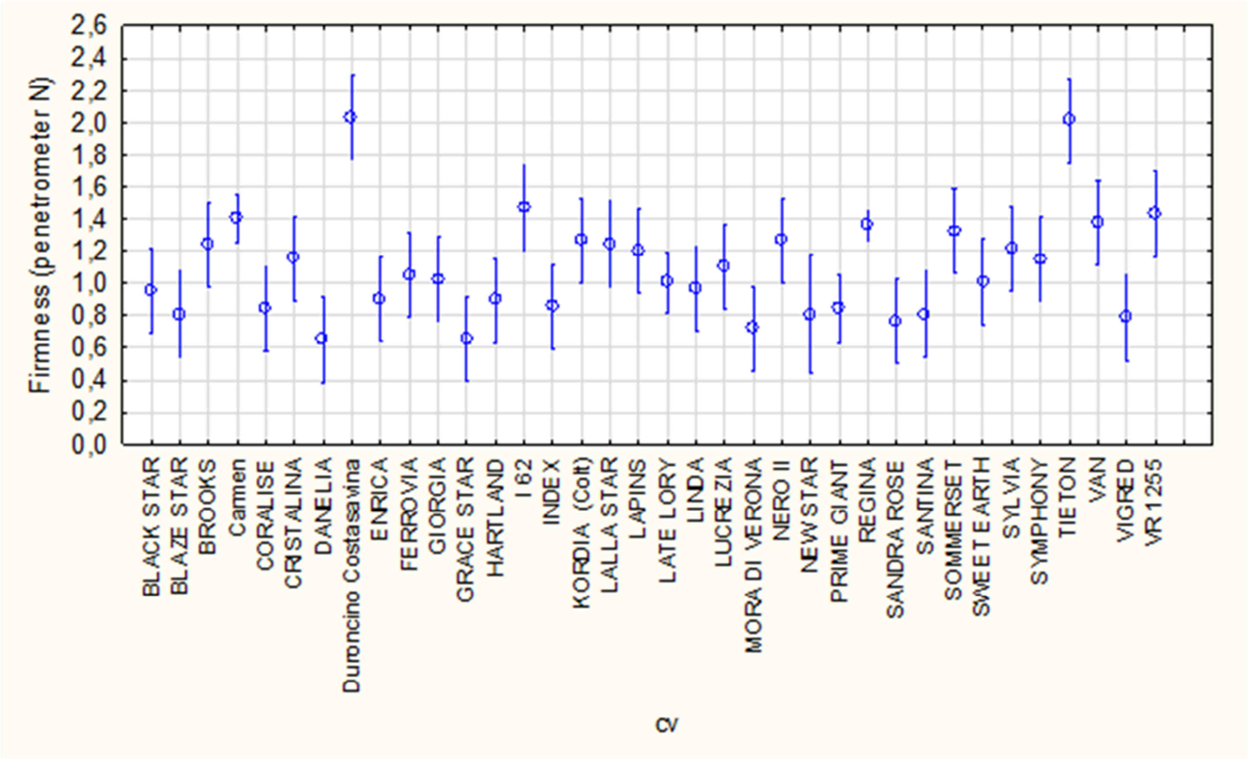

Figure 5.2. Results of the ANOVA analysis for the firmness trait measured by single point penetration on 36 cherry cultivars. Bars indicate interval confidence of 0,95. p=0,007

Texture variability was in general more informative than the single end point output of the penetrometer and allowed to better dissect the behaviour of the different tissue layers of the fruit to penetration. A general texture example of cherry texture profile is presented in Figg 5.3-5.4. Only one distinguishable force peaks is evidenced, which indicates the opposing resistance forces of the fruit when the probe penetrates the fruit anatomical structures. The profile is typical of a turgid round fruit, in which turgor plays a major role.

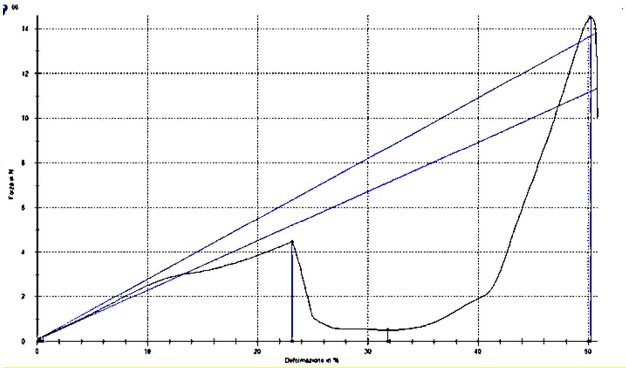

Figure 5.3 Example of cherry texture profile: Force (N) in the y-axis and strain in x-axis.

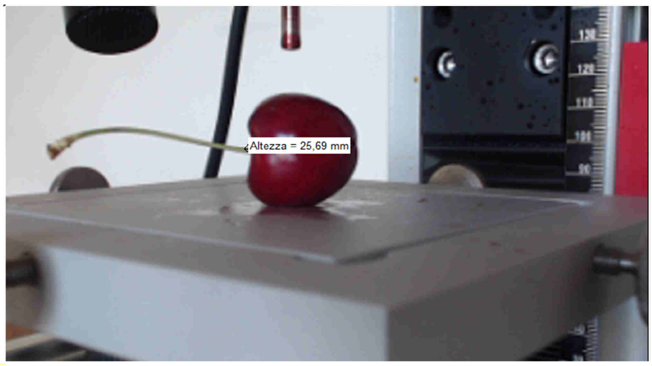

Figure 5.4 Sweet cherry fruit analysed by the texture equipment.

In particular, all the four textural parameters were significantly different with the cultivar. Concerning the parameter Gradient, which indicates the Young modulus or stiffness, is the ratio of tensile stress on tensile strain in the first elastic region of the fruit, which in the cherry anatomy is represented mainly by the epidermis and the first cellular layers of the mesocarp. The highest the Gradient value, the stiffest the tissue, thus less elastic and in the end “popping”. According to this parameter, values ranged from 0.9 MPa to 2.8 MPa, being the cultivars Tieton (2.8 MPa), Duroncino di Costasavina (2.5 MPa) the stiffest, while Index (0.9 MPa) is the most elastic.

Internal forces display a similar pattern, within a wider range of values, being the variability between 3 N and 11 N: if most of the cultivars show forces between 3 N and 5.5 N, like Duroncino di Costasavina (11 N) and Tieton (10.5 N). These two cultivars turned out to be both very compact in the internal and external layers, contributing to the definition of a very firm fruit.

Also the other two parameters, namely Distance and Area, showed a significant difference, and contribute to the definition of the profiling among cultivars.

The total variation of these data at harvest in the first year is explained up to 93 % (Fig. 5.5). PC1 (68 %) is mostly defined by forces parameters and Young’s modulus, while PC2 (25 %) explains the variation based on the distance linear force, orthogonal to the gradient. Duroncino di Costasavina and Tieton, which are the highest for Gradient too, follow the direction of forces, but still PC1 separates cultivars like Linda, Brooks, Kordia, Coralise quite clearly from the pool on the positive values of PC1 like Grace Star, Blaze Star, Lapins and Sweet Heart at harvest. PC2 indicates a separation of the cultivars mainly due to the Linear distance force. This parameter calculates the line joining all the consecutive points measured by the set strain of the probe entering the fruit. The highest the linear distance value, more difficult the fracture of the sample. Coralise expresses the highest values for this trait and Sweetheart the lowest.

At harvest though, the cultivars distinguished by negative PC1 and negative PC2, is characterized by stiff varieties with a high internal force, such as ‘Tieton’ and ‘Duroncino di Costasavina’. The second group (negative PC1 and positive PC2 negative quadrant) is composed of firmer and less fracturable cultivars, such as Coralise, Ferrovia and Linda. The third group (both PC1 and PC2 positive) is characterized by a cloud of cultivars with higher elasticity and deformable structure due to low internal forces, which may lead to the perception of gumminess by the consumer, like Index and Grace Star. The perception of a gummy fruit, is associated with a lower turgidity and a high resistance against the force required to break the superficial skin tension, increasing the strain of the maximum force, or compression value, at which berry disruption occurs. The fourth group (PC1 positive and PC2 negative) consists of a number of varieties, presenting low firmness and high elasticity values like Blaze Star, Lapins, Sweetheart, Sylvia and Durone Nero II.

In contrast with Muscovics et al. (2006), none of the firmness and texture parameters was significantly correlated with color measurements, thus color cannot be predicted from firmness and texture when many varieties are evaluated.

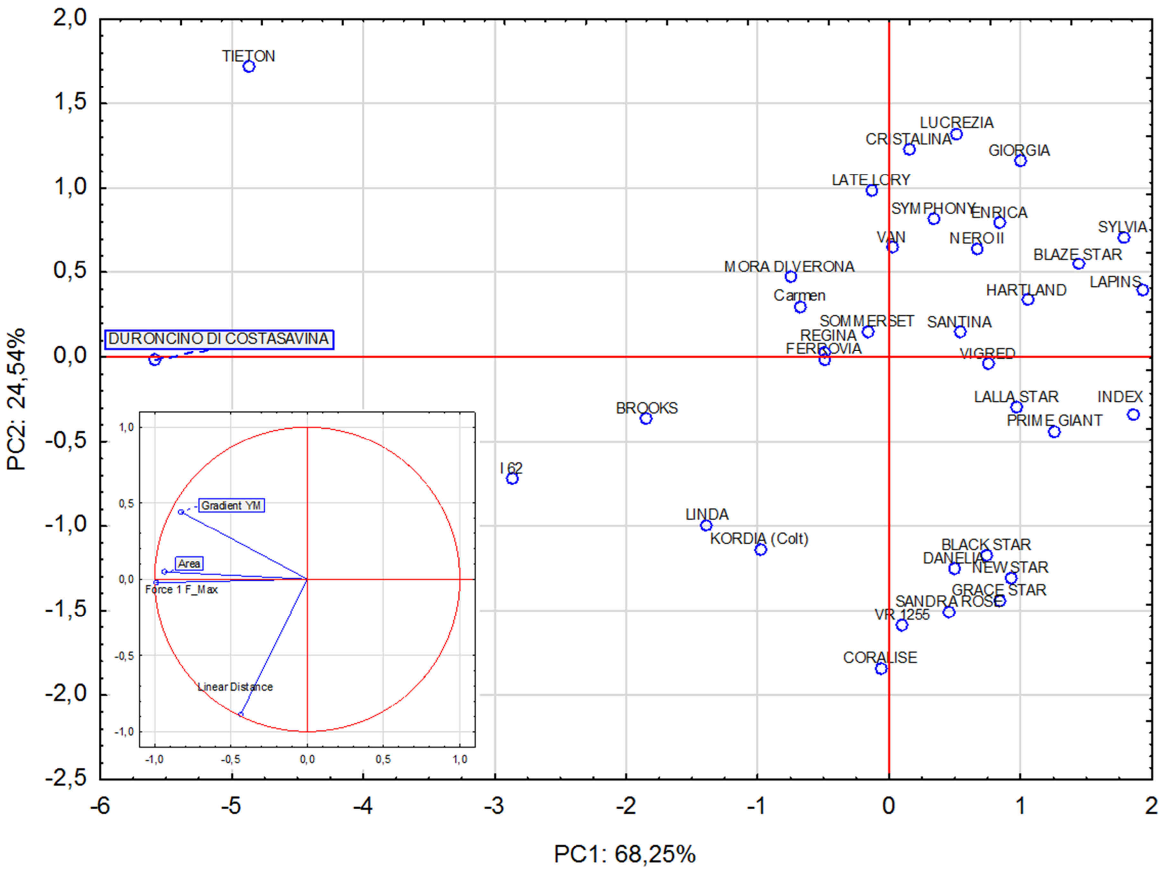

Figure 5.5. PCA representing the distribution of the sweet cherry cultivars at harvest dissected according to texture mechanical parameters and respective loadings.

The evaluation of the 36 cultivars over two years at harvest gave information on the stability of the parameters characterizing the trait and results are presented in Fig. 5.6. Over the two years, both firmness by the use of penetrometer and texture (4 parameters) were significantly different, but while firmness showed no cultivar significant differences, texture among the 36 cultivars was significantly different over the two years both for F_max and for Area. Gradient and linear distance force were not significantly changing, indicating a stability of the expression of the trait from the year of cultivation. In the PCA, the orientation of the vectors was consistent with the previous analysis, and the distribution allowed to understand, through a linear subtraction of the two principal components, of the stability of the cultivars. Late Lory was the most influenced by the year, followed by Tieton, Lucrezia and Sweet Heart. Kordia, Carmen and Lalla Star showed higher stability of the texture, for all the parameters analysed. The same genepool analysed for firmness showed a significant variation over the two years but not significant for the cultivar: this increases the importance of a more precise phenotyping methodology that allow discerning the different components of resistance to forces of the fruit. It seems in fact that the more external cellular tissue layers of cherry are consistent over the years, thus the Young’s modulus is stable, while more internal tissues, measured by the F_Max or the Area can change significantly. This can be due to a different osmosis with different cultivating years that contributes to a different retention of internal water, thus turgidity, that however seems to be independent from the skin and/or outer parenchymatic cells.

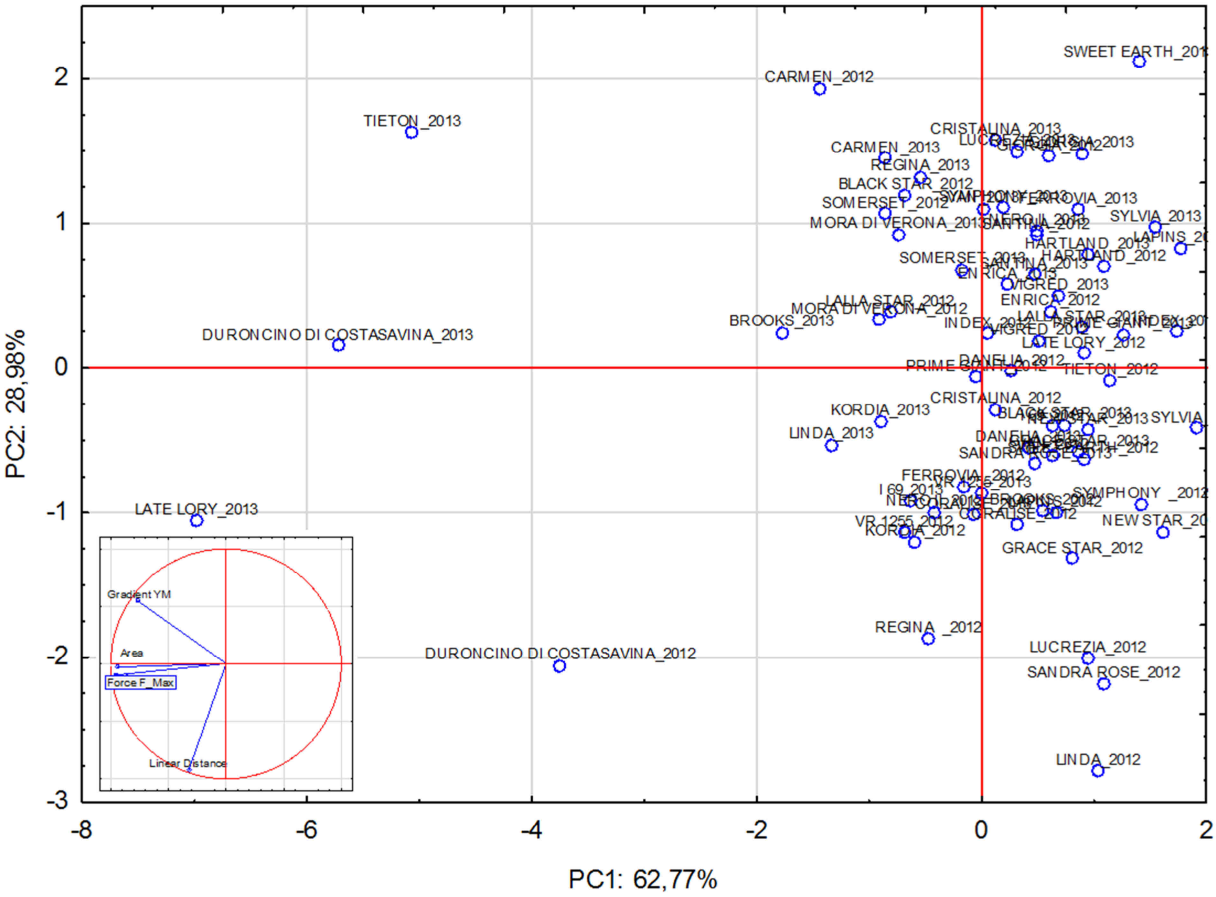

Figure 5.6. PCA representing the distribution of the sweet cherry cultivars at harvest over two years dissected according to texture mechanical parameters and respective loadings.

Texture comparison after postharvest and storage index

In modern worldwide marketing, fruits are regularly shipped and delivered from one hemisphere to the other, and to ensure high quality standards over the entire process the postharvest capacity is a fundamental prerequisite. In order to investigate the texture performance over postharvest, a second assessment was carried out on the set of cherry cultivars stored for two weeks at 4°C. The analysis of the texture variability in post-harvest, represented by a 2D-PCA plot (Fig. 5.7) illustrating the cases and B showing the variables) defined by the first two principal components accounted for 95% of the total variance and showed a consistent variable orientation as observed at harvest, with the two variables Gradient and Force closer each other. The reproducibility of the parameter projections, validated the robustness of the texture model presented here for cherry. In postharvest, a central cloud of varieties is very dense, but some varieties for the texture different parameters can be easily identified.

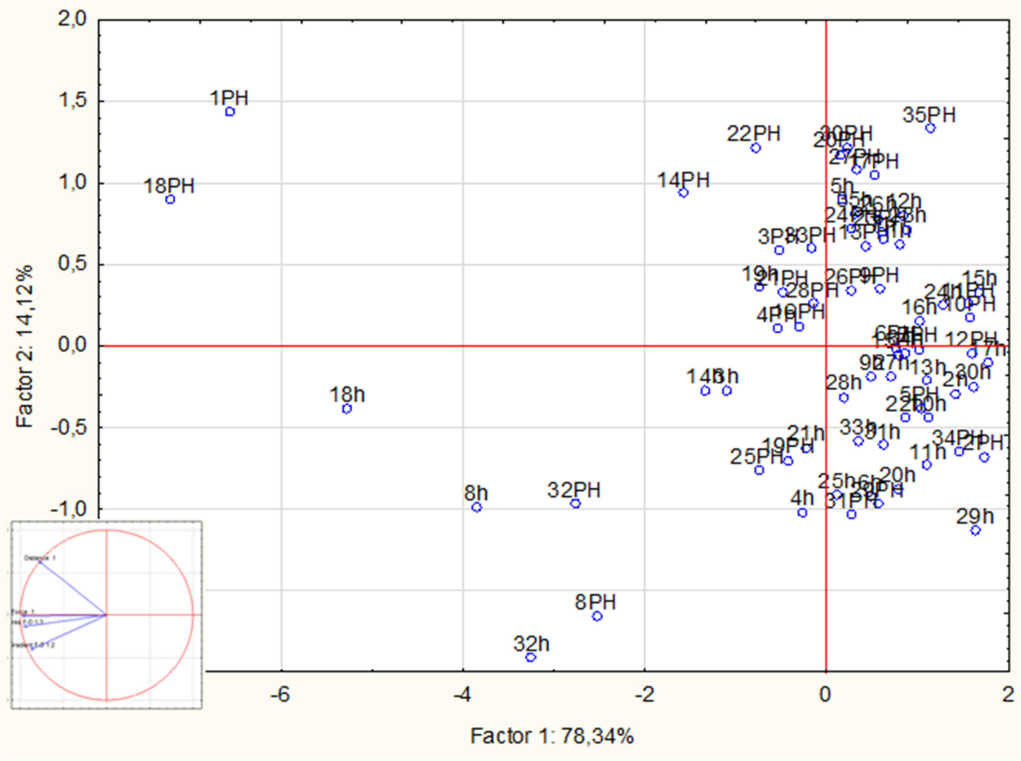

Figure 5.7. PCA representing the distribution of the sweet cherry cvs after storage (PH) compared to harvest (h) according to texture mechanical parameters and respective loadings.

The difference between gradient and internal firmness is quite low after harvest: the physiological modifications in the berry occur equally in the outer and inner layers and the low difference of the epidermic contribution at harvest is only marginally changing after harvest. In the PCA quadrant characterized by negative values for both PCs, none of the cultivars is outstanding compared to the others, although ‘Splendid’ and ‘Late Lory’ are plotted towards the area and firmness projection, showing high firmness values. Duroncino di Costasavina, Late Lory and Tieton in postharvest show a positive Gradient and high internal forces. Black Star is the highest after two weeks of storage for linear distance force, thus improving texture from a commercial point of view from harvest due to cold application to post harvest. This change was shown also in other crops like blueberry (Giongo et al., 2018). Cultivars like Enrica, Regina and New Star show, on the contrary, low changes in postharvest conditions for the texture parameters.

In order to have a broad and more exhaustive view of the fruit texture evolution during storage, the storage index was employed here on a set of cherry cultivars (Fig. 5.8), to describe the potential storability of each cultivar. The storage index, originally defined to investigate the fruit texture dynamics in blueberry (Giongo et al., 2013), provides valuable information related to the variation magnitude for each texture parameter during two months of storage, rather than an absolute value. This index, in fact, is the visual representation in a logarithmic scale of the ratio between these two stages (harvest and postharvest) for each assessed parameters, sum to each other. The illustration of the storage index, computed over the sample set, clearly showed a relevant variation for the sub-traits. The storage index illustration showed that cvs characterized by a minor change in elasticity during postharvest, are the most suitable to preserve a favourable texture performance throughout storage. In contrast, are also evident cultivars that showed a dramatic change in fruit turgidity, possibly caused by a physiological degradation process occurring during storage. These cultivars are thus less suitable for storage because of possible high fruit postharvest losses. The storage index (SI) provides information related to the magnitude of the variation for each parameter, rather than an absolute value. The storage index of cherry (Fig 5.8) indicates variation for the different texture sub-traits, revealing thus specific texture profiles clearly patterned for post-harvest attitudes of the different cultivars.

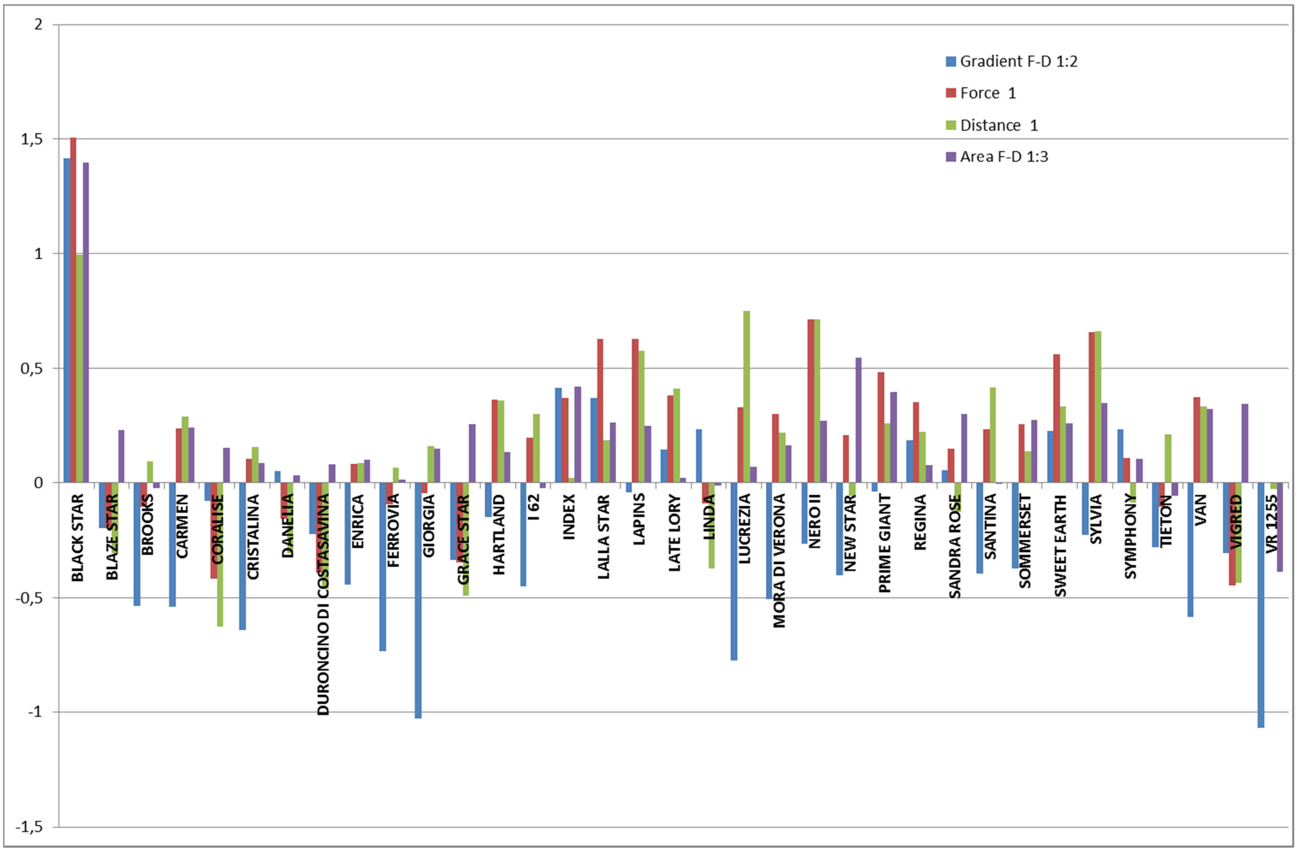

Figure 5.8. Results of the Storage Index calculated over the two timepoints (harvest and postharvest) for the individual parameter for the 36 sweet cherry cultivars.

Generally, all the parameters related to forces have a positive dynamic, while the Young's modulus evolves in a depletive way. This is not true for Black Star, which shows positive values for all the parameters, Regina, on a lower extent, Sweet Heart, Late Lory and Lalla Star. This can indicate a good suitability among cultivars to preserve a favourable texture performance over two weeks of storage and strengthening the importance of knowing the parameters that better contribute to cherry texture profiling.

Rootstock effect on sweet cherry texture

The influence of the rootstock on the texture of the scion fruit was analysed on the cvs Kordia and Regina. It is reported that a correct combination of scion × rootstock can produce fruits with higher firmness, weight, sugars, vitamins, and phenolic compounds that boost the fruit antioxidant activity (Correia et al., 2017). The different rootstocks have different characteristics, as resumed in table 5.3.

Table 5.3 Main characteristics of the rootstocks evaluated for texture on the scions Kordia and Regina.

ROOTSTOCK	CHARACTERISTICS
Colt (*P. avium* x *P. pseudocerasus*)	Semi-dwarfing rootstock. It is sensitive to droughty soils and cold winter temperatures. Vigour induced is similar to seedling. Somewhat slow to bearing. It has shown resistance to *Phytophthora* root rot and gopher damage, but is susceptible to crown gall caused by *Agrobacterium tumefaciens*.
Gisela 3 (*P. cerasus* x *P. canescens*)	Medium low vigor induced. Trees show sensitivity to replant stress. Some regenerative ability of root system is reported.
Gisela 5 (*P. cerasus* x *P. canescens*)	Medium low vigor induced with very high fruit production can cause fruit size and quality issues, a problem accentuated when it is combined with productive cultivars. It tends to advance both flowering and fruit ripening by two to four days and produces a tree that is open and spreading with wide branch angles, though branching may be sparse. Good winter hardiness. Trees show sensitivity to replant stress. Strong regenerative ability of root system is reported.
Gisela 6 (*P. cerasus* x *P. canescens*)	Medium to high vigor. Precocious, need to be properly pruned from an early age. In order to maintain fruit size and quality. Trees are open and spreading with good branching. Well suited for a wide range of soil types from light to heavy.
MaxMa (clonal selection of *P. mahleb* and *P. avium*)	Good graft affinity. Medium vigour induced. Suitable to all soil types. It induces low vigour. High yielding and early bearing.
Weiroot 158	Intermediate size. Good precocity, although somewhat less than Gisela 5. Fruit size is good.

Weiroot 72	Lowest vigor. Trees are very precocious and fruit size is slightly small. It requires excellent soil and optimum tree, soil, nutrition and water management to maintain fruit quality.
PiKu 1 (Complex hybrids).	Good graft affinity. Medium to low vigor. Suitable to moist and irrigated soils. It induces low vigour.
P-HL-C (*P. avium* x *P. cerasus*)	Good graft affinity. Medium to low vigor. Suitable to moist and irrigated soils. It can show poor root anchorage and require staking. Early bearing.

Selection of an appropriate graft combination is cardinal for the production of cherry, because the scion–rootstock interaction influences water relations, leaf gas exchange, mineral uptake, plant size, blossoming, timing of fruit set, fruit quality and yield efficiency (Schmitt et al. 1989, Nielsen and Kappel 1996, Gonçalves et al. 2003). Intensive canopies need dwarfing rootstock that can differently affect fruit quality and texture as well. Kordia and Regina are the two more commercial cultivars in Northern Italy, thus the comparative effect of Colt, Gisela 3, Gisela 5, Gisela 6, PHL-C, Piku 1 and Weiroot 158 was evaluated for the four parameters identified to dissect texture in cherry. Firmness and texture parameters varied with rootstock combination for the two cultivars. In particular, Kordia showed a better result of the Young's modulus on Gisela 6, contributing to a more turgid fruit, while on Gisela 5 the fruit tended to be more elastic and the Gradient lower. Regina performed better for the more external tissues on Gisela 5. Maximal force was not affected by the rootstock in both varieties, while LDF was significantly higher for Kordia on Weiroot 158 and Piku 1, while for Regina, this resulted on Gisela 5 and Gisela 3 (Fig. 5.9).

Although fruit quality is not usually the main evaluation of the different rootstocks' performance, it is clear from these results, that fruit quality, and texture in particular, is highly affected by the rootstock chosen for a specific scion. This might be explained by the different uptake of water in the fruit, due to a different development of the vascular system on the interface scion-rootstock and consequently of the ability of different roostock to supply water to the scion (fruit) (Goncalves et al., 2006).

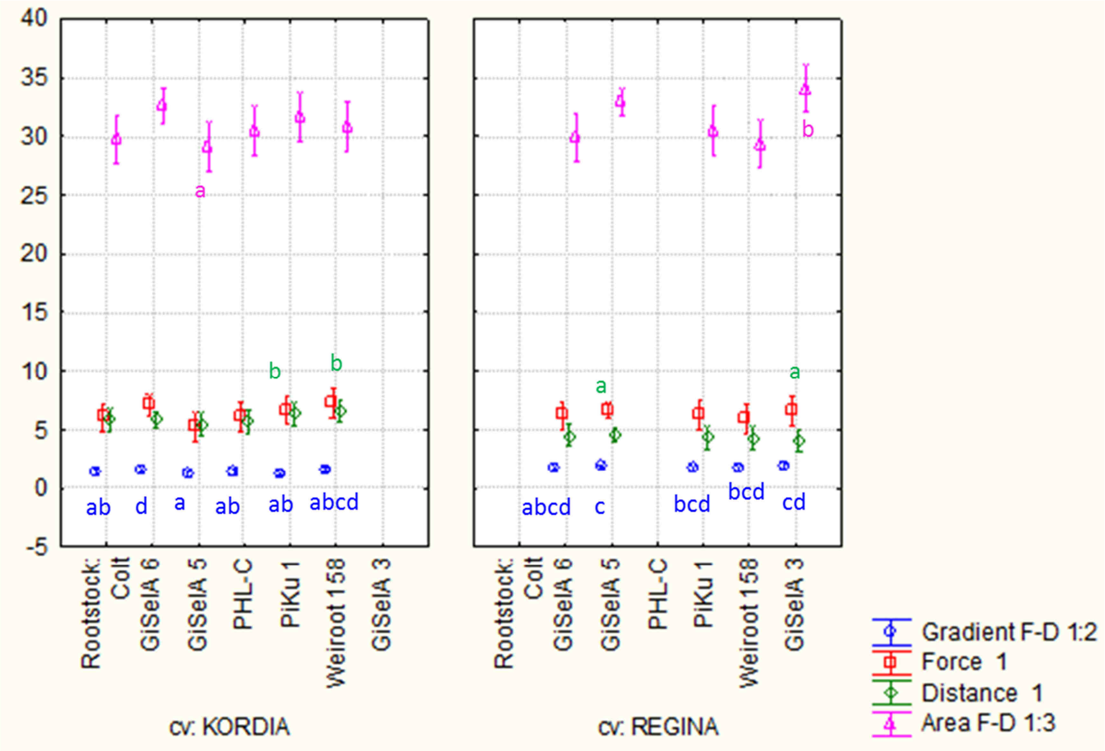

Figure 5.9. Results of the ANOVA calculated for the four mechanical texture parameters of Kordia and Regina on different rootstocks. Bars indicate interval confidence of 0,95. p=0,007. Letters indicate significant differences according to Tuckey *post-hoc test* (color is related to the respective parameters' color). Maximum force (Force 1) showed no significant differences.

Sensory panel test

Correlation between sensory scores and analytical data were elaborated through linear regression analysis for the different attributes. Chroma Index is weakly correlated with a general acceptability in terms of sensory score. Sugar content is even weaker and not significant, as well as aroma (3%), which probably results of difficult interpretation by the untrained panelists. When objective data are correlated with specific texture liking, the significance become stronger. The penetrometric data (single end point) results in a correlation of 32% with texture liking. Little correlation results between texture liking and maximal force (6%) or linear distance (2%). The correlation becomes significantly higher when plotted with the Young’s modulus (56%) and the area (21%).

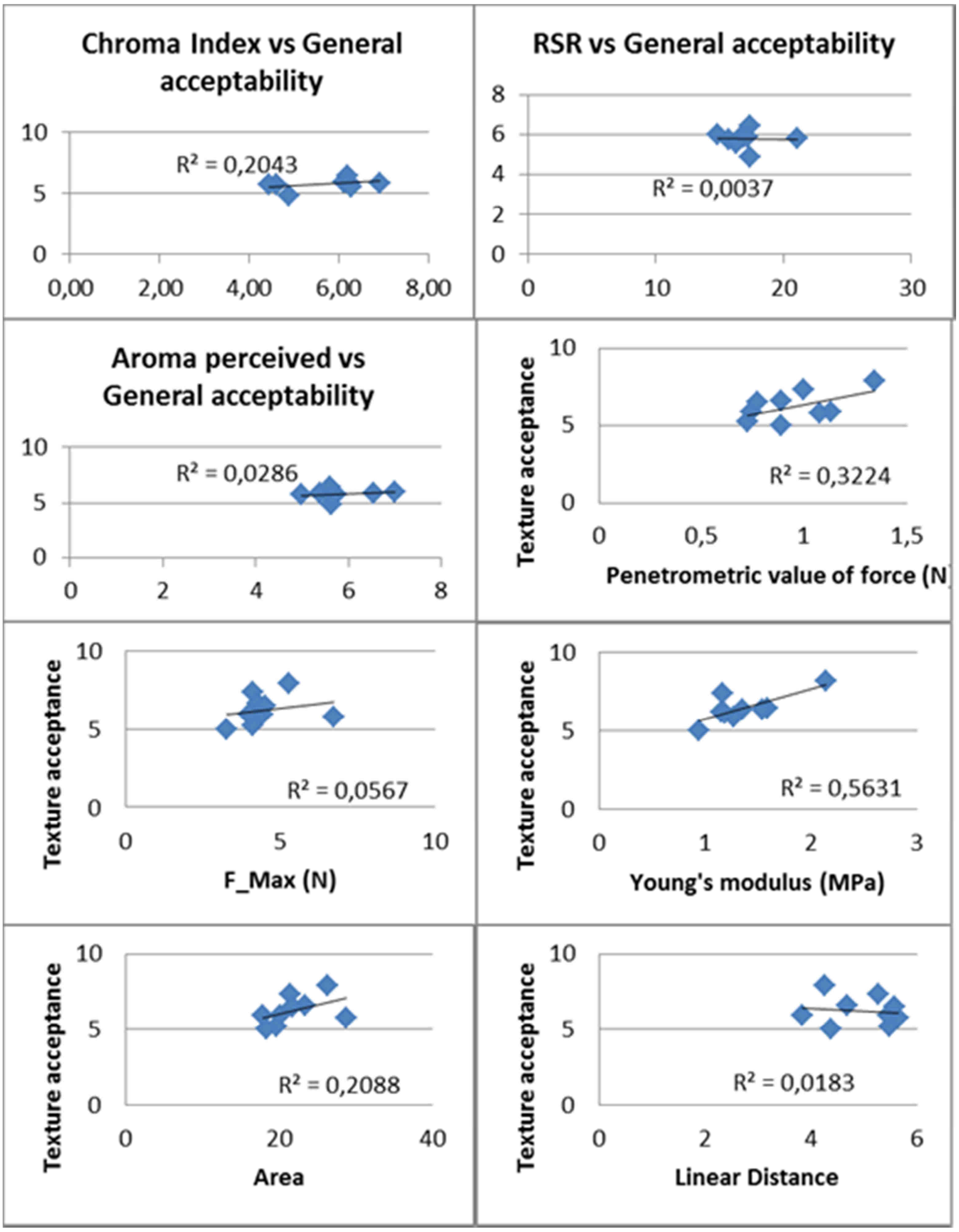

Figure 5.10 Visual interpretation of the correlation between sensory and attributes. y-axis represents in all the graphs the sensory scores (1-10) while x-axis is specific for sensory or analytical values. R^2 indicates correlation according to linear association.

The most influential instrumental parameters were identified for the different sensory characteristic through PCA. While the evaluation was previously conducted plotting two variables a time, by the use of the PCA all the variables are measured together on all the samples. The PCA explains 76% of the variance among the 9 cultivars evaluated, which separate according to the direction of all the different sensory and objective variables (loadings plot).

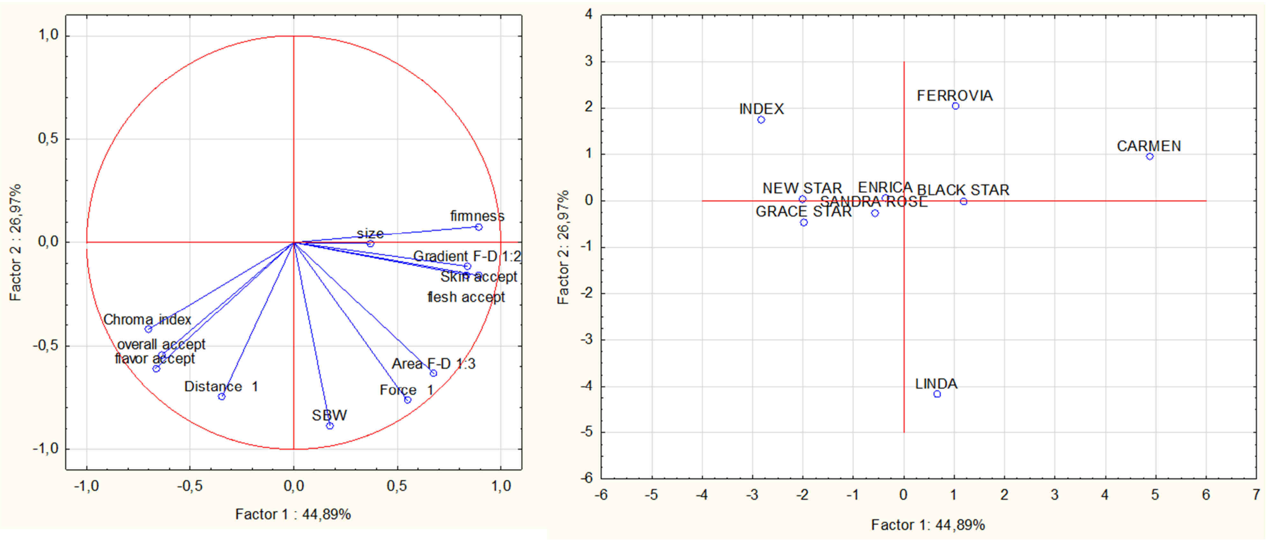

Figure 5.11 PCA representing the main attributes and objective values discriminating the nine cultivars evaluated and respective loadings (left chart).

In general, on the two right quadrants of the PC1, which contributes for about 45% of the variation, the samples are separated mainly according to firmness, Young’s modulus and acceptability of the skin and pulp of the sample. Carmen and Black Star belong to this group. On the left PC1 quadrants New Star, Grace Star and Sandra Rose plot close together. PC2 is mainly explained by single berry weight and overall acceptance: Linda shows positive values for these descriptors, while Ferrovia shows an opposite behavior.

It is interesting to notice that skin and flesh/pulp liking highly correlates with the gradient (Young’s modulus), which might thus be a robust predictor of liking and consumers’ acceptability.

CONCLUSIONS

Sweet cherry fruit shows among varieties a high degree of variability of the texture trait, together with other morphological traits that are quality-related. Fruit size is in general negatively correlated with firmness (Sam et al., 1999), although in this study this showed variability among cultivars. All the four textural parameters were significantly different with the cultivar at harvest and post-harvest. Texture variability was in general more informative than the single end point output of the penetrometer and allowed to better dissect the behaviour of the different tissue layers of the fruit to penetration. Also the storage index of sweet cherry indicates variation for the different texture sub-traits, revealing thus specific texture profiles clearly patterned for post-harvest attitudes of the different cultivars. Gradient and linear distance force are the two more stable parameters over two years of evaluation, while more internal forces can change more. This is in accordance with the study of Sekse (1995), which linked the cherry cracking to the nature of the fruit cuticle that acts as a semipermeable membrane in this process. External tissues, like cuticle, seem to be less influenced by the environment across the different genotypes than internal forces. Texture analysis allows indeed too precisely profile this component. The penetration analysis used in this study was a destructive one, because the need to anchor successive parameters that may be developed through compression for example was prioritary. Non-destructive methods of texture analysis however exist and they will be employed furtherly.

The storage index of sweet cherry here developed indicates variation for the different texture sub-traits from harvest to postharvest, revealing thus specific texture profiles clearly patterned for storage attitudes of the different cultivars. Finally, rootstock choice on specific scion cultivars affects texture as a component of fruit quality: this might be explained by the uptake of water in the fruit, due to a different development of vascular system on the interface scion-rootstock and consequently of the ability of different roostock to supply water to the scion (fruit). All together these texture related information can contribute to better assess this important trait, highly contributing to sweet cherry quality.

GENERAL CONCLUSIONS

Improving fruit quality is a major issue for berries and sweet cherry, strongly affecting marketability, and consequently the economic impact of new varieties.

To date, fruit texture modifications in berries have been mainly assessed with end point measurements, either by the use of penetrometers or FirmTechII, which infers firmness by compression. For a better evaluation of pulp texture evolution during the fruit development, we tailored for blueberry, raspberry, strawberry and sweet cherry a methodology for a more comprehensive texture dissection through a texture analyzer. The parameters and indexes identified in this work allowed the analytical characterization of texture sub-phenotypes: in particular, they are discriminant in distinguishing stage and genotypic specificity, in a validated and robust way.

Clearer, objective descriptors for crispiness, gumminess, elasticity, cohesiveness, stiffness, hardness and firmness were inferred with texture analysis to be used as biomarkers in seedlings selections. Some of these textural attributes are often associated with the freshness and firmness of fruit: crispness is described as the most versatile single texture parameter of a product that is universally liked, associated with pleasure and described as appealing (Tunick et al., 2013).

In berries and sweet cherry, this research highlighted that texture gradient (Young’s modulus), which has been subjectively estimated by breeders until now using their personal experience, maximal force, deformations, area and final forces are keys in the interpretation of the texture traits. The availability of these objective parameters allows a more precise description of fruit quality, particularly convenient for parents’ phenotyping and selection in breeding. Combining the profile of texture, aromatic compounds and morphological traits identified for the chosen germplasm, during fruit development, at harvest and during post-harvest was implemented especially for blueberry. All the information can enhance and focus the available tools for selection and its value in the selection process of breeding was shown as a linear incremental regression in the blueberry breeding program for different quality traits. According to our knowledge, this research represents an advancement in phenotyping: texture analysis able to reveal high genotypic differences and variability of texture in the berries germplasm of blueberry, raspberry, strawberry and cherry. Germplasms analysed are wide and representative, including 46 cultivars of blueberry and different progenies populations, 29 raspberry cultivars, 87 strawberries and 36 sweet cherry - and this reinforces the robustness of the output. Although still destructive in most of the analyses conducted, a part from raspberry, and not high throughput, texture analysys coupled with other analysis (eg: secondary metabolites, carpometric data) may allow to objectively measure a consistent portion of what is considered fruit quality.

The parameters can also become advantageous biomarkers to be used to segment, according to texture characteristics, different berries and sweet cherries into potential market categories according to texture characteristics at harvest for the fresh market or in post-harvest for their potential storage texture-related attitude.

The research highlighted that the two critical time-points, when fruit is harvested ripe and after post-harvest have necessarily to be considered separately when evaluating the genotypic attitude for taste development and selection. The dynamic modifications of texture traits in postharvest are highly significantly genotype depending. The ability to clearly identify the evolution of the parameter such as Young’s modulus which is highly informative of external damage symptoms for mechanical deformation, can, indeed, help to adopt different strategies to predict shelf life attitude and to reduce postharvest losses.

In addition to post-harvest, also pre- harvest factors influence texture: this study allowed to measuring the variability of the traits according to the year of evaluation for strawberry and sweet cherry and in this crop also according to the rootstock-scion interaction. While some texture parameters is strongly affected by the environment, others are stable under different treatment, suggesting an interplay of different genomic regions that should be furtherly investigated on the light of this new fine phenotyping results.

Furthermore, the output indicated also other fruit-environment interactions, since it is known that both cuticle characteristics and specific compounds are highly involved in pathogens' tolerance (Seymour et al., 2013) as well as environmental damages (eg: fruit cherry cracking due to rainfalls). The choice of the most suitable textured genotypes might beneficially influence cultivar tolerance in respect to both fungi and insects.

In perspective, texture analysis on berries and sweet cherries should be further widened: sensory analyses need to be coupled with the produced results in order to advance the information, as well as genetic and genomic studies on these traits. Microscopy and physiological data can also reinforce the quality texture profiling, as well as being able to monitor other quality parameters here not included, like juiciness, or to develop alternative non-destructive methods like acoustic and optical approaches (Chen et al., 2013) that can provide 'real-time' or 'on-line' texture measurement for fresh and processed foods. Exploring the germplasm and new progenies for new textures aromas and other fruit quality traits can be more feasible in the view of these results, but it might be enriched with other secondary metabolites profiling, in order to better explain the metabolic network (Bonghi et al. 2011) and specificity.

Due to the current lack of international standards for berries and sweet cherry texture measurements and fine phenotyping, this research on texture can contribute to define parameters settings able to profile, trace and compare quality components on these fruit categories, beneficial to breeders, producers, intermediaries and consumers.

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