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# SENSORY AND INSTRUMENTAL PROFILING OF APPLES: A NEW TOOL FOR QUALITY ASSESSMENT

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**Esame finale anno 2014**

*A Nonno Antonio.*

*Da persona lungimirante e appassionata*

*hai insegnato a noi nipoti il valore della cultura.*

*Grazie.*

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# *Chapter I*

# AN OVERVIEW OF SENSORY QUALITY OF APPLE FRUIT

## THE PERCEPTION OF QUALITY IN APPLES

Perceivable quality of a horticultural product is strictly linked to its freshness. Freshness is considered the key factor that determines consumer preferences in fruit and vegetable purchases (Ragaert et al., 2004). A fresh product is defined by UNI EN ISO 7563:1998 as “a turgescent product with no signs of withering or ageing, the cells of which have not deteriorated”. Since “texture” is defined by UNI EN ISO 5492:1992 as “all the mechanical, geometrical and surface attributes of a product perceptible by means of mechanical, tactile and, where appropriate, visual and auditory receptors”, it is easy to conclude that texture properties are the main factors responsible for freshness and for related consumer choice (Péneau et al., 2006; Harker et al., 2008). It is important to realize that texture consists of a number of different properties, not a single one, perceived by means of human senses and that its definition implies a sensory evaluation (Bourne, 2002). Texture analysis is used by the food industry, in fact, to define and check physical properties of food products, through the use of mechanical and rheological measurements. If such measures are to accurately predict sensory perception of texture parameters, human assessment should be the standard against which instrument readings should be calibrated. In this way, it would be possible to have a product which falls within the range of textural parameters that experience has shown to be acceptable to the consumer (Bourne, 2002; Harker et al., 2003).

Fruit shape, size, color, soluble solids content, acidity and firmness are the parameters most considered for defining apple quality standards. Compression measurements by penetrometry are the most widely used technique for firmness evaluation (Harker et al., 1997; Qing et al., 2008). Sensory analyses, instead, are not usually considered for general quality assessment of fruit. However, in the case of fruit like apples, texture properties are not dissociated from other properties, such as olfactory and gustatory ones, and consumer preferences are generally based on a combination of texture and flavour (Daillant-Spinnler et al., 1996; Harker et al. 2003; Gatti et al., 2011). These relationships justify a sensory-based approach as the starting point for implementing measurement tools that are effective in predicting human perception of apple quality.

## METHODOLOGICAL ASPECTS

### *Sensory profiling*

Sensory analysis is the only approach able to provide a direct evaluation of sensory properties and an overall product profile, rather than studying just one attribute at a time. In addition, it is also suited to giving an objective meaning to sensory perception, in qualitative and quantitative terms. Sensory analyses, in fact, have the aim of describing products in an objective way, characterising them by scientific criteria, and defining perceivable differences (Murray et al., 2001). For these reasons, sensory analyses requires scientific competency and appropriate facilities, such as laboratories specifically equipped for performing sensory tests. Although sensory analyses use a scientific approach, they are able to explain perceivable quality of food by using a language which is close to that of the consumer (Swahn et al., 2010; Seppä et al., 2012).

Descriptive analysis is the most sophisticated of the sensory methodologies available. It requires a panel of trained judges to score the intensity of a series of specific attributes of a product on a linear or numerical scale. The result of such analysis consists of a complete description of sensory properties of one or more products that are related to appearance, odour, flavour and texture. Moreover, it provides the basis to map similarities and differences and to highlight which sensory attributes are important to consumer acceptance (Stone and Sidel, 2004a).

Until 1996, there were no studies that established that the sensory properties evaluated through instrumental tests could actually represent the attributes which are really important for consumer choice (Daillant-Spinnler et al., 1996).

In general, not all studies applying sensory analysis to apples report details about the sensory methodologies that were employed: some aspects, such as vocabulary development, panel selection and judge performance, were often not sufficiently described and discussed to be fully understood. In most studies the attributes were chosen by a brain-storming among the judges (Daillant-Spinnler et al., 1996; Allan-Wojtas et al., 2003). In other cases, the sensory vocabulary was proposed by the panel leader (Karlsen et al., 1999; Harker et al., 2002a, 2002b; Harker et al., 2006). In particular, studies focused on the relationship between sensory and instrumental data often proposed a specific set of sensory descriptors which might fit with the sensory meaning given to the instrumental measures. Hence texture sensory properties might have been defined for physical measurements or flavour attributes for volatile compounds analysis (Karlsen et al., 1999; Ioannides et al., 2007; Chauvin et al., 2010). Many studies referred to ISO standards for general sensory analysis methodologies and panel selection (Karlsen et al., 1999; Echeverría et al., 2008), whereas Daillant-Spinnler et al. (1996) provided a detailed description of panel training, specific for apple profiling.

One of the few papers providing detailed information about panel performance evaluation was by Hampson et al. (2000), who proposed a tool to analyse judge consistency and performance over several years. Accuracy of sensory data is of fundamental importance: if sensory data are not reliable, i.e. consistent and discriminant, with a good agreement among the judges, sensory profiles are not reliable and any prediction models can show low effectiveness.

An important consideration comes from the work by Brookfield et al. (2011), who focused on explaining the wide range of different correlations between sensory and instrumental data that can be observed in the literature, and concluded that such variability probably depends on the different cultivars tested in each study – different cultivars tend to respond in a different way to the various models that are used. Their conclusion suggests that a very large set of apple cultivars should be considered in such studies in order to cover the range of variability that can occur within different apple properties.

### ***The link to the consumer***

After defining a product sensory profile it is necessary to identify which attributes are important to the consumer and in which way.

With regard to the sensory definition of freshness, the main attributes used by the consumer to judge apple freshness are crunchiness, juiciness and mealiness. The first two of these are considered to be positive factors, while the last one is negative (Péneau et al., 2006; Oraguzie et al., 2009).

Crunchiness is an acoustic attribute, evaluated as the intensity and frequency of the sound produced by chewing (Duizer, 2001; Fillion and Kilcast, 2002). Juiciness is associated with a tactile sensation; it represents the juice amount released by the product during chewing (Harker et al., 2002a; Ioannides et al., 2009). Mealiness is a qualitative defect, appearing as dry and “sandy” flesh which breaks down into fine particles as consequence of the weakening of intercellular bonding. In mealy apples, fractures occur as a result of cell-to-cell debonding, and individual cells do not break to release their contents (Harker et al., 2006; Echeverría et al., 2008).

Harker et al. (2003) highlighted an important feature that needs to be considered when studying apple preferences, that is, acceptability defines different consumer clusters that are characterised by preferences towards different sensory profiles. For example, it is possible to distinguish people who like crisp and sweet apples from others who like juicy and sour fruit. Often, specific groupings of preferred attributes are the result of expectations related to experience. Since clusters of genes associated with fruit quality usually change together, consumer preferences tend to link specific taste and texture properties because they are generally associated in different cultivars (Harker et al., 2003). The differences in preferences can also be related to consumer age (the elderly tend to like softer and more sour apples; vice versa for children), or to nationality or ethnic group, determining a higher or lower familiarity towards different products (Prescott and Bell, 1995).

Wills et al. (1980) were among the first to study consumer liking and its relation to sensory properties. Daillant-Spinnler et al. (1996) studied the relation between sensory properties perceived by a trained panel and consumer preferences for different apple varieties. Texture and taste properties were considered more important by the consumers than aroma and appearance. Nevertheless, the relation between preferences and sensory profiles was not the same for all the cultivars: some of the cultivars appeared to be quite different based on sensory properties but very similar in terms of consumer preferences. The authors concluded that it is not possible to define a sensory property-based methodology useful to predict acceptance in absolute terms (Daillant-Spinnler et al., 1996). Jaeger et al. (1998) tested the hypothesis that consumers perceive apple mealiness as a negative attribute and show a higher preference for fresh apples, rather than stale ones. Fresh apples were evaluated as harder, juicer and crisper by a trained panel, while stored apples were described as old, stale and floury. The consumer test, in contrast with the results by Daillant-Spinnler et al. (1996), showed that the first dimension on the preference map was strongly related to flavour properties while the second dimension was related to texture differences. However, the conclusion was the same: although the trained panel highlighted perceivable differences related to storage treatment within each variety, acceptance appeared to be more strongly linked to the variety factor, irrespective of the mealiness level (Jaeger et al., 1998). Recently Bonany et al. (2014) performed a consumer preference test on several apple varieties in seven different European countries. They defined an external preference map relating the consumer preferences to the sensory profile described by a trained panel and to instrumental characterisation, suggesting such a tool as useful for the positioning of the variety in the market and for leading breeding activities. However, even if sensory description and instrumental characterisation seemed to be well related, the authors stressed that it is not a simple task to interpret the results coming from preference tests in order to define practical standards of quality (Bonany et al., 2014). Moreover, Seppä et al. (2013b) found that the initial liking or disliking expressed by consumers toward an apple cultivar did not always reflect their final choice, since that choice was often influenced by other options the consumers had during the selection process. This result demonstrated that expressed preferences are not to be considered as a constant, but they are strongly dependent on the context.

### *Relationship between sensory and instrumental data*

Although the importance of sensory analysis is unquestionable, these methods are expensive and time consuming and, for these reasons, these analyses are not always suited to practical use when many samples need to be analysed. It is, therefore, desirable to replace sensory evaluation by faster, simpler, or cheaper instrumental analysis. For these reasons several studies have examined correlations between sensory and instrumental data.

### *Texture parameters*

Firmness is the most considered and studied texture parameter. In the study by Harker et al. (2002a), instrumentation tests showed that a minimum difference of 6-8 N in instrumental firmness with an 11mm probe puncture test was necessary to have a difference in sensory attributes perceived by a trained sensory panel. Below a minimum value of 50 N measured by the firmness test, the fruit were evaluated as being mealy by the trained panel. So, it is possible to define a critical puncture threshold, below which the apples are described as being mealy, and apple producers could define a threshold in their practical measures to ensure that mealy apples are excluded from a pack-out (Harker et al., 2002a). Chauvin et al. (2010), found a logarithmic relationship between physical properties of apples and the sensory scores determined from descriptive analysis, and reported that when apples are soft, humans are more sensitive to textural differences than instruments are. When apples are hard, the ability of panelists to perceive differences may decrease because of fatigue; thus, in this case, instrumental determination would be more reliable than the panelists' (Chauvin et al., 2010). Nevertheless, *in vivo* measurements of texture properties proposed by Ioannides et al. (2007), by means of electromyography (EMG; that records facial muscle activity during apple chewing) when compared to penetrometry analyses, showed that penetrometry was only able to replicate the first bite, without providing information on the tissue modification that takes place in the mouth as a result of the chewing process. That factor was considered by the authors to be a limitation of penetrometry in providing effective data for predicting texture sensory properties (Ioannides et al., 2007). However, a limitation of psychological origin in the EMG tracing does exist: the volunteers tended to chew in a different way when they were asked to evaluate some sensory attributes, rather than responding normally when there would be less stress and less need to concentrate (Ioannides et al., 2009).

Several authors have focused on the acoustic parameters. Apples, like all fresh vegetables, are composed of living cells, with cell walls fastened to each other by means of the middle lamella and subjected to turgor pressure, which is higher than the external atmospheric pressure. The breaking of the cell wall provokes the rapid expansion of the liquid content, responsible for the sound emission. Acoustic emission amplitude and frequency are strictly related to the perception of crispness and crunchiness, which are very complex concepts, combining a wide range of perceptions, such as sounds, fracture characteristics, density and geometry (Fillion and Kilcast, 2002). Study of consumer responses demonstrated that crispness is characterised by a sudden, clean fracture occurring when a crisp food is bitten. The noise emitted is perceived to be higher pitched and louder than the sound produced during biting crunchy foods, showing low pitch sounds and characterised by a certain degree of bone conduction. That is why the combination of acoustic and mechanical techniques more adequately describes food acoustic properties perception than either technique alone (Duizer et al., 2001). De Belie et al. (2002) studied the acoustic parameter of crispness that had been separately scored by a trained sensory panel by combining measurements taken by a microphone of the sound emitted during chewing of a sample coming from the same fruit. A fundamental limitation was the use of different subjects and different samples from the same fruit for sensory and instrumental measures: subjects involved in sensory analysis were not the same subjects involved in chewing recordings. The authors proposed that a better relationship between chewing sound and sensory data might be expected if the recordings were taken from each panelist as he/she was scoring for texture attributes

(De Belie et al., 2002). Crispness and crunchiness have important cognitive implications: Demattè et al. (submitted) demonstrated that artificial modifications of specific frequencies of the sound perceived in real time during biting or chewing of apples significantly affects crispness perception, demonstrating that crispness is an attribute strongly related to the acoustic information coming from the food. Hardness perception was also found to be affected by sound modifications, although it is defined as a mechanical attribute, showing a multisensory interaction in hardness perception. Zdunek et al. (2010a) developed a contact acoustic emission detector, based on the simultaneous use of a puncture test and an acoustic emission detector in contact with the sample during the test. They found that total acoustic emission counts were a better predictor of texture sensory attributes evaluated by a trained panel than penetrometry firmness measurements alone, particularly with respect to crispness, crunchiness and hardness (Zdunek et al., 2010a). Costa et al. (2011) related mechanical and acoustic data recorded on apple samples during compression by a texture analyser to the texture sensory evaluation by a panel of experts. They found that the instrumental acoustic parameters were positively correlated to sensory crispness and negatively to firmness, suggesting an important role of acoustic parameters in the perception of crispness. Hence high crispness and high firmness were not dependent on each other and it should not be expected that they would be present together in any case (Costa et al., 2011).

The relationship between apple tissue anatomical features and texture properties has been studied by several authors (Allan-Wojtas et al., 2003; Mann et al., 2005; Billy et al., 2008). Allan-Wojtas et al. (2003) compared the sensory description of apples by a trained panel with a micro-structural analysis of the flesh matrix by microscopy. By defining groups of apple cultivars with common sensory profiles and studying the structural properties representative of each group, they were able to describe the structural components responsible for specific sensory responses (Allan-Wojtas et al., 2003). Mann et al. (2005) correlated apple anatomical features and texture sensory properties, finding that cell number was important to the prediction of crispness and mealiness, suggesting that fruit with a fewer number of cells per unit area were crisper than fruit with a higher number of cells per unit area, while cell size predicted juiciness, suggesting that bigger cells release more juice (Mann et al., 2005). Useful interpretations come from Ting et al. (2013), who used X-ray tomography to study the anatomical features of different apple varieties and their relation to instrumental firmness. They found that different microstructural organization and the distribution, number, and size of intercellular spaces were responsible for different texture properties that were characteristic of different apple varieties. The work by Billy et al. (2008) found a relationship between texture sensory profile and water-soluble pectin (WSP) extraction analysis: mealiness and “fondant” attributes were positively and negatively correlated, respectively, to the concentration of galacturonic acid in the WSP extract.

### *Flavour parameters*

Several authors have found difficulty in developing effective predictive models for taste in apples based on predicting flavour sensory perception from instrumental measures of compositional data. The main reason seems to be the multisensory nature of taste perception, characterised by interference from other sensory properties.

Harker et al. (2002b) found a good prediction for acid taste by titratable acidity, while soluble solids concentration showed a poor relation with perceived sweetness. These authors asserted that assessment of fruit by sensory analysis should remain a critical part of fruit quality assessment, since sweetness represents one of the most important factors affecting consumer liking (Harker et al., 2002b). Additional studies highlighted that influences between different sensory properties exist that are able to affect sweetness perception. Harker et al. (2006) demonstrated that sweetness perception depends on the degree of breakdown of apple flesh during chewing – i.e., it depends on textural properties – rather than on differences in sugar and acid content (Harker et al., 2006). Echeverría et al. (2008) found a relation between sweetness and mealiness perception scored by a trained panel, with high mealiness values being related to low sweetness values, even if no real correlation between the two sensory attributes was found. Another interesting conclusion from this work was that a low consensus in the panel was observed for those attributes having high interactions with others, e.g., sweetness (Echeverría et al., 2008).

The influence of other sensory properties can also be observed with aroma perception. Karlsen et al. (1999) looked for a correlation between sensory data and instrumental data coming from texture and volatile compounds (VOCs) analysis on several apple varieties. The highest correlations were obtained when sensory odour and flavour attributes were correlated at the same time to texture and VOCs instrumental data – the prediction of aroma perception seems to require information about apple texture properties. Differences in flavour release could be due to structural differences as every compound responsible for flavour has to be released from the apple matrix to come in contact with taste and olfactory receptors. Release kinetics are therefore influenced by the chewing process, the interaction with saliva, and mouth temperature, which depend both on apple and on subject characteristics (Foster et al., 2011; Chen and Engelen, 2012). Moreover, Aprea et al. (2012) found that the interaction of the same volatile compounds when present at different concentrations can be responsible for the perception of different perceived odours or flavours. Ting et al. (2012) showed that nose-space proton-transfer reaction mass spectrometry analysis of volatiles released during apple consumption provides significant information about real flavour perception. They found that very different volatile profiles came from apple fruit during chewing, as compared to *in vitro* VOC measurements on the same apple cultivars, confirming that nose-space analysis provides data that better explain real consumer perception.

The general conclusion is that it is possible to obtain a better sensory attribute prediction if a larger number of instrumental and/or chemical measurements are taken into account when elaborating a model (Karlsen et al., 1999).

### *Overall profile*

Non-destructive techniques have also been developed and applied to study overall apple quality, since resulting spectra developed from chemometric techniques can give a general overview of a product profile which can be used to predict sensory properties.

Mehinagic et al. (2003) tested the effectiveness of vis/NIR spectroscopy in predicting sensory properties. They found that mealiness was negatively and crispness positively correlated with spectroscopic data in the wavelength range corresponding to chlorophyll and starch absorbance bands. Chlorophyll and starch are subjected to changes in their concentrations during ripening, which is a process that also involves structural modifications indicating why a relation between vis/NIR measures and some textural attributes might exist. Sweetness was negatively correlated and sourness positively correlated with absorbance at wavelengths corresponding to starch. Starch degradation during ripening is the basic mechanism for sugar production, responsible for sweet taste, while, concurrently, acid concentration tends to decrease. Despite these interesting results, the relationships were not strong enough in comparison with the better correlations observed between sensory data and penetrometry measures (Mehinagic et al., 2003). Rizzolo et al. (2010) used time-resolved reflectance spectroscopy (TRS), a technique which measures concurrently the absorption coefficient and the scattering coefficient at different wavelengths – the absorption coefficient is a measure related to the absorption of photons by pigments (chlorophyll, carotenoids) and by main chemical components of the flesh (water, sugars), while the scattering coefficient is a measure related to photon refractive mismatch caused by cellular structures, such as membranes, cell walls, intercellular spaces, starch granules, etc. The authors found good correlations between texture sensory attributes and some scattering coefficients. Sweet taste showed a significant correlation with some absorbance coefficients. The authors were optimistic about the ability to predict texture sensory attributes, mealiness in particular, by TRS. However, the best correlations were found between sensory scores and other more common destructive measurements used as the control.

In conclusion, non-destructive techniques (vis/NIR, TRS) seem to be promising in the prediction of some sensory attributes, but are not yet as reliable as commonly used destructive analytical methods.

### *Consumer preferences*

Some authors have studied the relation between consumer preference and instrumental characterisation, as a direct way to interpret preferences in terms of chemical and physical properties.

Hoehn et al. (2003) compared consumer preference with chemical and mechanical measures on apples. The authors found how not only soft apples, but also very hard ones were not preferred by consumers, even by the youngest. Such observations confirm the theory that liking falls within a range of intensity for each sensory characteristic (Bourne, 2002). Similar to other studies, they found a good correlation between instrumental measures and liking for one apple cultivar, but not for others. According to the author, this finding should be taken into account when defining the minimum tolerance standards for the instrumental parameters used for quality assurance applied to apples – an instrument is not able to measure the same combination of properties that human senses can, and several sensory attributes together can influence preference judgment (Hoehn et al., 2003). In this context, Harker et al. (2008) tested the instrumental measurements currently available for quality control in order to verify whether they provide appropriate quality parameters to define consumer acceptability. In their work, an increase in liking was found when firmness measured by penetrometry was above a specific threshold common to all the varieties examined (‘Gala’, ‘Red Delicious’, ‘Fuji’ and ‘Braeburn’) and equal to 62 N. The authors observed that the market success or failure for an apple cultivar can depend on the ratio between the cultivar’s natural firmness distribution and the firmness threshold below which consumers reject apples. When the proportion of fruit below that limit is high, the variety tends to be less appreciated and purchased (Harker et al., 2008).

## APPLICATION OF SENSORY ANALYSIS IN APPLE STUDIES

The study of apple quality includes a series of factors that need to be considered, such as the impact of growing conditions; post-harvest storage conditions and physiological changes during storage; post-storage shelf conditions; and properties and peculiarities of new cultivars being released from breeding activities. In the light of the established important role of sensory science in the evaluation of apple quality, it is important to consider the application of descriptive sensory analysis and preference tests in determining the significance of such factors.

### *Pre-harvest factors: some examples*

Crop management practices and pre-harvest treatments are able to influence product quality both at harvest and during storage, mainly in terms of cell anatomy, structure and turgor (Sams, 1999; Johnston et al., 2002). Many studies are available about the influence of factors such as rootstocks, irrigation and fertilization management, weather conditions, and canopy structure on apple fruit yield and quality, measured in terms of instrumental parameters (e.g. fruit weight, firmness, soluble solids concentration, disease and pest damage, and the incidence of physiological disorders;

see, for example, Racsko et al., 2008; Campi et al., 2009; Brackmann et al., 2010; Casero et al., 2010; Lachapelle et al., 2013).

However, few studies relating pre-harvest factors with quality determined by fruit sensory analysis are available. In terms of growing practices, Vanzo et al. (2013) compared apples produced by organic and integrated systems. A consumer panel performed triangle tests and hedonic evaluation of specific sensory attributes. The results showed that consumers were able to discriminate between fruit coming from the different growing systems and that the preferences between organic and integrated fruit for sweetness, tartness, firmness, juiciness, overall flavour and appearance were cultivar dependent. Altitude is also a factor determining differences in ripening stage and fruit chemical composition (Comai et al., 2005; Singh et al., 2006; Aslantas and Karakurt, 2007). Paprštein et al. (2006) studied fruit chosen from orchards in four climatically different locations (about 200, 300, 400 and 500 m a.s.l.) by asking panels of consumers to score their liking for several sensory attributes related to appearance, flavour and texture. The authors reported a total score, representing the sum of scores for each attribute, and a general taste score, but they did not perform any statistical analysis to study the differences in sensory properties of each cultivar at the different locations and no evidence of significant differences related to altitude was provided.

Crop load is also known as a factor affecting fruit quality and sensory properties. Baugher and Schupp (2010), for example, demonstrated better quality, in terms of sensory profile and consumer liking, in fruit coming from low crop load treatments compared to high crop load treatments in ‘Honeycrisp’ apple. Thinning is therefore a key factor to improving crop yield and quality in apple (Link, 2000). The most used way to reduce crop load in apple is the application of phytochemicals which cause fruit abscission (Zibordi et al., 2009). An innovative method consists in shading apple trees by appropriate nets (Byers et al., 1990) – competition for reduced photosynthates is responsible for fruit abscission (Corelli Grappadelli et al., 1990). There are conflicting results about the final quality of fruit coming from shading treatments (Widmer, 2008; Zibordi et al., 2009; Amarante et al., 2011). Recently, photoselective colored shading nets have been proposed to promote specific physiological responses by differential spectral transmission of solar radiation (Shahak et al., 2004). Bastías et al. (2012) found small instrument-measured differences in apple fruit coming from trees under different colored nets. Solomakhin and Blanke (2010) also found that sugar/acid ratio, indicative of “taste”, was not influenced by photoselective net treatments, probably because of the tendency of sugars and acids to decrease in the same proportion in all the treatments (Solomakhin and Blanke, 2010).

In light of the observations reported here about the importance of sensory perception and the definition of ranges of acceptability for several quality parameters, a consideration of eating quality just based on sugar/acid ratio appears not adequate to reliably describe the quality of fruit. To our knowledge, no studies applying sensory analysis to evaluate the quality of apples coming from different thinning practices and different photoselective net treatments have been published yet.

### *Post-harvest changes of apple sensory properties*

One of the first studies applying sensory analysis to study post-harvest changes in apples was proposed by Watada et al. (1980), who found strong differences in the sensory patterns for five apple varieties developed during a five-month storage period, and suggested that this might be due to differences in physiological age at harvest. Some varieties, for example, showed high astringency at harvest, typical of unripe fruit. For such cultivars, there was a strong change in their sensory profile during storage, more than in other cultivars which could be indicative of that fruit being more ripe at harvest. However, the authors did not ignore potential differences in chemical composition and cellular structure, suggesting the usefulness of studies on anatomy or metabolic and catabolic processes, determining the relationship between these factors and sensory quality (Watada et al., 1980). Several authors have found that different apple varieties exhibit different patterns in both sensory texture and flavour profiles during storage (Billy et al., 2008; Seppä et al., 2013a). Seppä et al. (2013a) defined clusters of varieties, depending on their sensory profile, and found that most of them moved from one cluster to another during storage as their sensory properties changed. Hence, different varieties can show similar sensory profiles at a specific moment during storage but very different profiles at another. Billy et al. (2008) explained the different patterns exhibited by different varieties during storage as related to different genetic profiles and different enzymatic metabolism of pectins (Billy et al., 2008).

Modifications in sensory properties during storage of apples do not seem to be related only to textural properties, since it has been demonstrated that volatile compound release strongly changes during post-harvest storage, and that different patterns can be shown by different apple varieties (Soukoulis et al., 2012). Aaby et al. (2002) found that differences in sensory properties between fresh and stored apples were mainly related to odour and aroma, while texture and taste attributes did not differ significantly, even if instrumental firmness and titratable acidity decreased during storage (Aaby et al., 2002). Varela et al. (2005; 2008) studied the relation between changes of sensory profile of apples during storage, evaluated by a trained panel, and consumer acceptability. Rejection of fruit was associated with increased mealiness, ripe and alcoholic flavour, even if other attributes (such as juiciness, sweetness, acidity) remain unchanged. Thus, attributes that are most often considered important did not influence the decision by consumers to reject the fruit (Varela et al., 2005). They also highlighted the fact that fruit recently harvested and fruit stored in either cold or controlled atmosphere conditions showed different patterns in how their sensory properties changed subsequently during storage at room temperature (simulating real market conditions) irrespective of similar instrumental parameters measured at harvest or soon after storage (Varela et al., 2008).

Other studies proposed instrumental measure analysis as a way to predict apple sensory quality change during post-harvest storage. Mehinagic et al. (2004) employed both descriptive sensory analysis and instrumental measures (penetrometry, compression test, vis/NIR spectroscopy, soluble solid and titratable acidity concentrations) to predict sensory properties at harvest and during storage. Penetrometry appeared to predict sensory properties well at harvest, while the compression test helped to better explain the changes in mealiness and juiciness after storage (Mehinagic et al., 2004).

An important conclusion from such studies is that different cultivars show different sensory patterns during post-harvest storage. That suggests the need to develop and validate sensory tools on very wide sets of apple cultivars, in order to define the different patterns that can be show within this genus. Moreover, cultivars could be studied under different storage conditions in order to enhance differences in their responses. Instrumental analyses could also provide information about the chemical and structural changes responsible for the different trends, as highlighted by Costa et al. (2012), who observed a considerable textural variation in texture analyser performance of different apple cultivars over two months of storage. Since the authors considered that the main source of variation was genetically based, they suggested that proper evaluation of apple storage performance should be considered as a basic factor in breeding programs so that varieties which can best maintain quality features during storage can be selected (Costa et al., 2012).

### ***Breeding studies***

Currently, the most advanced method of breeding is marker-assisted selection, based on the identification of individuals carrying gene alleles responsible for the phenotype of interest (Costa et al., 2010a; Sansavini and Tartarini, 2011; Myles, 2013). Preliminary screenings made on the initial wide set of breeding progeny are necessary, before any sensory characterisation, in order to reduce the samples to a number which can be managed in sensory evaluations. However, such preliminary instrumental screenings can exclude interesting cultivars, because of an improper transposition of instrumental readings in sensory interpretation. Thus, the implementation of reliable prediction models for apple sensory quality by instrumental measures is required if they are to be applied in breeding studies. Even where disease resistance and facilitating of efficient growing practices are among the most important targets to breeders, new apple selections must also have a high appeal to consumers and this makes the description of their sensory characteristics all the more relevant.

Within this context, a number of sensory studies have been concerned with determining consumer acceptability of new apple genotypes. Granger et al. (1992) studied new scab-resistant apple cultivars for their sensory acceptability through hedonic evaluation of different quality attributes using a flavour profile technique (Caul et al., 1958). The overall acceptability of each apple variety was calculated as the difference between the average score for positive quality attributes (aroma, sweetness, acidity, firmness, juiciness and crispness) and the average score for negative quality attributes (astringency, bitterness and mealiness). A five-year study by Paprštein et al. (2006) on the acceptability of more than a hundred cultivars currently cultivated in the Czech and Slovak Republics together with new promising ones, harvested in four climatically different locations, aimed at identifying which climatic condition could be proposed as being the best for achieving the best sensory quality score for each cultivar. A similar study was conducted by Miller et al. (2005), who studied 20 new apple cultivars both in the eastern US and in British Columbia, Canada. Hedonic scales were used to score the liking for appearance, texture and flavour, while intensity scales were used to score the intensity of texture and taste attributes. Significant differences in apple sensory quality were found for cultivar and site. The authors suggested that widespread sensory tests of new apple cultivars across several sites should always be considered in order to evaluate new apple cultivar performance under different soil and climatic conditions (Miller et al., 2005).

Bonany et al. (2013) tested products grown in a specific site and then tested by different consumers at different locations around Europe. The results showed significant interactions between apple variety and country, age and gender, indicating that differences in eating quality acceptance among varieties were influenced by these factors. A sensory profile developed on the same fruit by a trained panel provided a definition of those sensory characteristics that were appreciated in different countries and by different consumer classes (Bonany et al., 2013). The first work that applied concurrently descriptive analyses and consumer surveys was performed by Redalen (1988) on about 35 new apple selections over a five-year period. The study was mainly centered on flavour characteristics and appearance, resulting in conformity between the highest scores for the intensity of flavour properties given by the trained panel and preferences expressed by consumers. However, no regression analyses were proposed in that study to explain and confirm such a relationship (Redalen, 1988). Hampson et al. (2000), instead, developed a more detailed protocol for the definition of liking drivers on new apple varieties. Firstly, a trained panel was involved in the hedonic evaluation of seven sensory attributes related to appearance, texture and flavour of both new cultivars and of standard varieties, over a period of four years. A consumer preference test was then performed on a sub-set of samples. The authors found out that crispness accounted for 90% of variation in texture liking and sweetness, sourness and aromatics explained about 50% of the variation in flavour liking. They also performed instrumental mechanical and chemical measurements on the samples, but found that the collected sensory data were better predictors of liking than the instrumental methods were. Thus, the authors' conclusion was that analytical measurements are not adequate to substitute for sensory evaluation in screening new breeding products

(Hampson et al., 2000). Kühn and Thybo (2001), instead, applied descriptive sensory analysis only, studying scab-resistant apple cultivars for their sensory properties by a trained sensory panel which assessed 13 different attributes. The cultivars, which were evaluated at different storage times, showed differences that were related both to cultivar and to storage time (Kühn and Thybo, 2001).

## CONCLUSIONS

This overview shows that the study of apple eating quality has been of interest for a long time, both in relation to sensory properties and to consumer acceptability. However, not all the available papers have reported stringent criteria for the use of sensory protocols. A wide series of studies also applied instrumental analyses to confirm sensory data and to interpret them, and also to identify correlations between sensory and instrumental variables to predict the sensory profile, with some common difficulties, as for the prediction of sweetness perception. Many studies can be cited as being methodological, as they report different and sometimes innovative sensory and instrumental methodologies to evaluate apple eating quality. The application of sensory analysis in specific studies on apple quality in relation to pre- and post-harvest factors, as well as the study of sensory characteristics of new varieties is, instead, not common in the literature, mainly because of the limitations of sensory methodologies, which require more time and specialized resources than instrumental characterisations.

However, there is still space for the development of proper sensory methodologies that can go hand in hand with instrumental characterisations, in order to define effective prediction models to provide apple producers with a reliable description of apple sensory profiles.

# *Chapter II*

# SENSORY PROFILING OF APPLE: METHODOLOGICAL ASPECTS, CULTIVAR CHARACTERISATION AND POSTHARVEST CHANGES

## INTRODUCTION

Eating quality is a key factor driving the choices of consumers in fruit and vegetable consumption (Harker et al., 2003) and largely depends on the fruit properties formed and established both at the end of the fruit ripening process and throughout postharvest ripening. Fruit ripening is a complex of physiological processes that makes the fruit edible and pleasant. The most important changes are in fruit size, colour, acid/sugar, flavour and texture. Texture, in particular, is a major attribute used for the determination of apple fruit quality because of its tight correlation with general fruit freshness. A fresh fruit is defined by ISO 7563:1998 as “a turgescent product with no signs of withering or ageing, the cells of which have not deteriorated”; thus the texture properties are recognised as the most important drivers for consumer acceptability (Jaeger et al., 1998; Péneau et al., 2006; Harker et al., 2008). In addition, texture characteristics related to mechanical and elastic properties of the primary cell wall structure, are also responsible for juice and flavour release, which are also important characteristics in determining apple fruit quality (Daillant-Spinnler et al., 1996; Karlsen et al., 1999; Harker et al., 2008).

The quality of apples is currently measured by food suppliers using basic pomological descriptors, such as fruit shape, size, colour, soluble solids content, titratable acidity and penetrometer measurements (i.e., the most widely used method for quality texture assessment) (Harker et al., 1997, Hoehn et al., 2003). Many studies have attempted to predict eating fruit quality using these instrumental characterisations (Harker et al., 2002a and 2002b Chauvin et al., 2010; Zdunek et al., 2010a). However, in some cases, the predictions have been too empirical because of the interaction among several sensory attributes, making the analyses of these chemical and physical properties insufficient for an exhaustive fruit quality description (Harker et al., 2006; Echeverría et al., 2008). Recently, a novel texture analyser was employed to obtain a comprehensive apple fruit texture characterisation while simultaneously profiling the mechanical and the acoustic texture components (Costa et al., 2011 and 2012). However, apple eating quality cannot be estimated on the basis of a single instrumental parameter but it must be analysed as a whole.

Descriptive sensory analysis is perhaps the best approach to provide a comprehensive and objective description of sensory perception in both qualitative and quantitative terms (Murray et al., 2001). Therefore, human assessment should be maintained as the main reference to calibrate any instrument to develop testing methods accepted by consumers (Bourne, 2002). Moreover, sensory analysis could help to describe the product’s characteristics using a language that closely reflects the consumers’ perception (Swahn et al., 2010; Seppä et al., 2012).

During last two decades, several protocols for sensory profiles of apple fruit have been proposed. Most of them focused on the relation between instrumental and sensory measurement (Dever et al., 1995; Harker et al., 2002a and 2002b; Allan-

Wojtas et al., 2003; Echeverría et al., 2004; Chauvin et al., 2010). Other protocols were developed for specific cultivars, studying their change during storage or after different postharvest treatments (Boylston et al., 1994; Cliff et al., 1998; Pre-Aymard et al., 2005). Some topics, such as vocabulary development, panel selection and judges performance, are often not sufficiently considered.

In the majority of the published studies the attributes were chosen by panel brainstorming, based on discussion about the meaning and the use of each sensory variable (Daillant-Spinnler et al., 1996; Kühn and Thybo, 2001; Allan-Wojtas et al., 2003); sometimes the vocabulary was directly proposed by the panel leader (Karlsen et al., 1999; Péneau et al., 2007; Harker et al., 2002a and 2002b). Scientific contributions interested in the relationship between sensory and instrumental data often propose a specific set of sensory descriptors which may fit with the sensory meaning given to the instrumental measures, such as texture properties for firmness measurements or flavour attributes for volatile compounds analysis (Karlsen et al., 1999; Ioannides et al., 2007; Chauvin et al., 2010).

As regard as the panel selection, many studies refer to various ISO standards for general sensory analysis methodologies (Karlsen et al., 1999; Kühn and Thybo, 2001; Echeverría et al., 2008), whereas Daillant-Spinnler et al. (1996) provide a more detailed description of panel training specific for apple profiling. In addition, Hampson et al. (2000) propose a way to monitor panel performance. Moreover, this literature has proposed several different types of sample presentation, from the whole fruit (Cliff et al., 1998; Seppä et al., 2012) that avoids alterations due to browning and allows a realistic external appearance evaluation, to half fruit (Karlsen et al., 1999; Harker et al., 2002a; Billy et al., 2008) or peeled/unpeeled single slices

(Daillant-Spinnler et al. 1996; Barreiro et al., 1998; Hampson et al., 2000; Péneau et al., 2007; Chauvin et al., 2010; Brookfield et al., 2011) or flesh cubes (Varela et al., 2008) that makes a sub-sample available for instrumental measurements.

Based on the results published so far, the aim of this work was 1) to develop a detailed and complete protocol for apple sensory profiling performed by a trained panel, from judges training and sample preparation to panel performance evaluation and method validation, 2) to apply this method to a wide selection of relevant cultivars in order to acquire information about their sensory properties and 3) to investigate the changes in sensory characteristics during postharvest storage.

Twenty-one different apple cultivars were chosen, the largest feasible set, including the most consumed ones on the Italian market and those used in previous studies conducted at Fondazione Edmund Mach (FEM) (Costa et al., 2011). Additionally, twelve cultivars in the apple set were also analysed after different postharvest storage periods to observe the modifications of the sensory properties during postharvest.

The evolution in fruit sensory quality during conservation is of fundamental importance for apples because these fruits are generally consumed after a period of storage (which can last for almost a year). Additionally, several apple varieties respond in a distinct and specific cultivar-dependent manner.

# MATERIALS AND METHODS

## *Plant Materials*

### *Apple sampling*

Twenty-one apple varieties (*Malus*×*domestica* Borkh.) were considered in this study (Table 1) and selected based on a previous study looking at the mechanical and acoustic profiles of a large apple collection (Costa et al., 2011). The most common commercial apple cultivars (‘Cripps Pink’, ‘Gala’, ‘Golden Delicious’, ‘Granny Smith’, ‘Fuji’, ‘Renetta Canada’) were included in this study.

The experimental design for cultivar characterisation included one sampling of each of the 21 varieties. For 6 of these (‘Braeburn’, ‘Cripps Pink’, ‘Fuji’, ‘Golden Delicious’, ‘Granny Smith’ and ‘Renetta Canada’), a second sample was considered. All the fruit were harvested in the year 2010 from experimental orchards managed according to standard agronomical practises (i.e., thinning and pest control).

The fruit were picked at commercial harvest, determined by the standard descriptors used to monitor fruit maturity and ripening, such as flesh firmness, skin colour, total acids, sugar content and starch degradation index. For each sample, a minimum of 20 apples of homogeneous size and without any visible external damage were selected and stored for two months in normal atmosphere at 2°C and 95% relative humidity. Furthermore, to follow the changes in sensory characteristics during storage, sample subsets from 12 varieties were assessed after 1 month (7 varieties) and 4 months (12 varieties; Table 1).

### *Sample preparation*

For each apple batch, 16 fruit were selected and kept at room temperature for 24 hours prior to analysis. Each fruit was weighed (Table 1) and then peeled. Three horizontal sections, 1.2 cm high each, were cut around the equatorial plane perpendicular to the core of the fruit. The slices were then immediately dipped in an antioxidant solution (0.2% citric acid, 0.2% ascorbic acid, 0.5% calcium chloride) for 30 seconds. Cylinder shapes (1.8 cm diameter, 5 or 6 cylinders per slice) were cut from the flesh using a commercial apple corer (Tescoma, Brescia, Italy). These flesh pieces underwent a second antioxidant treatment before being placed into clear plastic cups (8 cylinders per cup) with lids and encoded with a random three-digit code. Six apple samples were analysed per session, 3 varieties each with two replicates; sample identities were blinded and they were presented in a randomised balanced order to each assessor.

The juices squeezed from each cultivar (12 cylinders sampled from different fruit) were measured for % of soluble solids concentration (SSC) (DBR35 refractometer, XS Instruments, Poncarale, Brescia, Italy) and titratable acidity (Compact Titrator, Crison Instruments S.A., Alella, Barcelona, Spain) (Table 1).

### ***Sensory analysis***

#### *Panel selection and training*

The selected panel included 13 people: 6 males and 7 females. Eleven had previous experience in sensory analysis. Twenty-eight candidates, all employed at FEM, were initially evaluated based on their performance during a preliminary training. The training was performed in 6 sessions, each 1.5 hours in duration, through a teamwork, and 9 individual tests were performed that aimed to assess the ability of each candidate to recognise and measure the basic tastes (Table 2a) and several common odours (Table 2b; UNI EN ISO 8586-1; ISO 8586-2). The taste and odour stimuli were presented in water and commercial cloudy apple juice solutions (100% apple juice; Pfanner Getränke GmbH, Lauterach, Austria). For each individual test, 1 point was assigned to each correct answer given by the assessors, and the test scores were weighted for the total stimuli presented and then summed to compute the individual cumulative score. This score and the percentage of attendance were considered for the eligibility of a candidate to the panel, using a threshold of 60% and 80%, respectively.

#### *Sensory profiling*

Sensory profiling based on the quantitative descriptive analysis method was performed by the selected assessors (Stone and Sidel, 2004a).

A 15-attribute sensory lexicon was developed using the consensus method (Murray et al., 2001) over 9 training sessions. A specific and univocal sensory definition along with a precise evaluation procedure was agreed upon by the panel for each attribute (Table 3). The developed lexicon included attributes for external flesh appearance (2), texture (7), taste (4, comprising astringency) and overall odour perceived by both ortho- and retro-nasal evaluation. The intensity of each attribute was scored by the panel on a linear scale, anchored to 0 (minimum intensity or absence) and 100 (maximum intensity), with a third anchor at halfway (50). References were provided for each attribute, corresponding to the intensities at the scale extremities (Table 3).

The sensory tests were performed once per week in a sensory laboratory equipped with twelve individual booths under artificial lighting. Unsalted bread and still water were provided to the assessors to cleanse their palates between samples. Data acquisition was achieved through a computerised system using the software FIZZ 2.46A (Biosystemes, Couternon, France).

### *Statistical analysis*

To evaluate the consistency and discriminant ability of the assessors, ANOVA for each assessor and attribute was performed; the results were plotted on a *p*-value vs. Mean Square Error-value (MSE) plot (Næs et al., 2010). Panel consonance was evaluated using correlation loading plots based on the Tucker-1 method (Næs et al., 2010). Both analyses were performed with the PanelCheck V1.4.0 software (Nofima Mat, Technical University of Denmark and University of Copenhagen).

The product averaged sensory profiles were determined by univariate and multivariate approaches. One-way ANOVA was performed on the whole data set considering the cultivar effect; two-way ANOVA was performed on the subset of 7 varieties analysed after 1, 2 and 4 months of storage (see Table 1), considering cultivar and time of storage as factors. Effects with a *p*-value less than 0.05 were considered significant. ANOVA was performed using the STATISTICA 9.1 software (StatSoft, Inc., U.S.A.).

For visualisation of the product sensory space, Generalised Procrustes Analysis (GPA) was performed separately on both data sets using the Senstools 3.1.6 software (OP&P Product Research BV, Utrecht, the Netherlands).

## RESULTS AND DISCUSSION

### *Method validation*

#### *Panel performance and vocabulary validation*

Assessors' consistency and discriminant ability and panel consonance were evaluated on the complete data set (13 judges x 15 sensory attributes x 27 apple samples x 2 replicates).

The results of ANOVA for each assessor and attribute are summarised in the *p*-MSE plots, shown in Figure 1. The *p*-value calculated for a specific assessor and attribute indicates the ability of the assessor to distinguish one or more samples from the others. In contrast, the MSE represents the repeatability of an assessor's evaluation (all evaluations were conducted in duplicate). A good assessor should possess an ideal combination of low *p*-values and low MSE-values (Lea et al., 1995), as highlighted by a dashed area in Figure 1. All the assessors are located in this area for most of the attributes, except for those indicated by different symbols. The cases with *p*-value greater than 0.05 are related to the attributes overall odour, overall flavour, graininess and bitter taste. With regards to MSE, only one judge had values greater than the critical threshold (> 400) for the astringency and bitter taste attributes. The best results for all the assessors were observed for the crunchiness, flouriness and sour taste attributes.

The agreement among the assessors was studied using Tucker-1 correlation plots for each attribute. Figure 2 shows two examples of such a graph (for hardness and bitter taste). The two ellipses on each plot correspond to 50% and 100% of the explained variance. For a well-trained panel, the correlation loading of a specific attribute should be close to the outer ellipse, with the assessors plotted closely together (Næs et al., 2010). The application of Tucker-1 plots on our data showed that the best consensus among the assessors was obtained for all the texture attributes, sour taste and external appearance attributes. As an example of these results, Figure 2a shows the Tucker-1 plot performed on hardness attribute data: it can be noted how this attribute was used in agreement among the judges. Figure 2b represents the Tucker-1 plot for bitter taste attribute, in which the assessors showed a low correlation and less than 50% of the explained variance was achieved. A low consensus for overall odour was found as well and it is probably due to the lack of a more specific attribute definition, which might have allowed the assessors to reach a better agreement on its interpretation. A not very high correlation among the assessors for sweet taste and overall flavour could be due to the confounding effect of other parameters, mainly texture, which can interfere with sweetness perception and volatile compounds release from the fruit tissues during chewing (Karlsen et al., 1999; Harker et al., 2006; Echeverría et al., 2008).

In order to evaluate any possible effects of bitter taste sensitivity on individual performance in using this attribute, the judges' taster status has been investigated. Eleven out of thirteen judges were tested with PROP (6-n-propylthiouracil), according to the method proposed by Tepper et al. (2001). The results revealed that 54.5% of the judges were "medium tasters", 36.4% were "non-tasters", and only one judge (9.1%) was a "supertaster". Nevertheless, no relation between PROP sensitivity and reliability, discriminant capacity or consensus among the judges was highlighted. It is therefore possible to assume that the low performance observed could be due to the limited use of "bitter taste" attribute as extremely low values were assigned by the assessors for all the samples. Thus, since "bitter taste" descriptor was considered as not discriminant (as shown by *p*-MSE plot of Figure 1) nor reliable (as shown by Tucker-1 correlation plot of Figure 2b), it was excluded from the data-set.

A systematic screening was also applied on results coming from each weekly session (data not shown), in order to monitor the judges' performance: when problems were noticed for one judge on any attribute, the subject was invited to take part to a specific training session, to discuss again the use of attributes, definitions, reference standards and evaluation methods. This meticulous practice allowed maintaining the best agreement in the use of the sensory vocabulary among the judges, during the long work period they were involved in.

The evaluation of the panel performance, paying attention to the reliability and the use of each descriptor by each judge, is important to confirm the accuracy and the effectiveness of the implemented tool. This is the first time that a method based on statistical methods tailored for sensory data is applied to apple sensory profiling. Our results show the usefulness of such an analysis, allowing the removal of useless and confounding descriptors and the screening for ability of each component of the trained panel.

### *Sample preparation procedure*

Different methods for sample presentation are presented in the literature. Whole fruit (Cliff et al., 1998; Seppä et al., 2012) was considered not convenient for our study, since the use of some well-known varieties could easily provoke bias due to previous knowledge and experience by the assessors (Harker et al., 2003). Baugher et al. (2010) showed that fruit external appearance can be responsible for prejudices about other sensory characteristics, such as texture and flavour. Presumably, these prejudices may also influence the evaluation of new unknown apple cultivars coming from breeding activity, related just for shape or skin colour to other better known varieties.

Other studies proposed the use of half of a fruit (Karlsen et al., 1999; Harker et al., 2002a; Billy et al., 2008) or single slices (Daillant-Spinnler et al. 1996; Barreiro et al., 1998; Hampson et al., 2000; Péneau et al., 2007; Chauvin et al., 2010; Brookfield et al., 2011), but Dever et al. (1995) had previously demonstrated that different portions cut from the same apple could be evaluated as significantly different, due to differences between top/bottom or blush/non blush fruit sides.

Therefore, in our study, we decided to present samples cut in small equal flesh pieces, which may ensure that each judge can taste pieces from more fruit (8 cylinders sampled from 8 different fruit) and that each fruit can be evaluated by more than one judge. That would allow each sample to be a good representation of the variability present in the batch and the score by each assessor for each attribute to be closer to the real average for the product.

The sample preparation is quite longer than other proposed methodologies, but the dipping in an antioxidant solution allows the sample to be preserved for long time before the panel evaluation. Previous tests were performed to ensure that the antioxidant components were used in a concentration which does not modify the original taste properties (data not shown).

We think that an effective representation of the averaged sensory attribute intensities would highlight possible differences between fruit belonging either to the same batch analysed in different storage periods, or belonging to distinct parental genotypes used in breeding programmes.

### ***Cultivar sensory profiling***

One-way ANOVA on the descriptive sensory data shows the existence of significant differences among the different apple cultivars for all the sensory attributes, with a *p*-value lower or equal to 0.001. For the overall odour attribute, a difference was found only between ‘Granny Smith’ and ‘Golden Delicious’ varieties (data not shown).

Figure 3 shows the GPA bi-plot of the sensory data for the 21 assessed varieties, with the first two dimensions explaining 47.6% and 18.8% of the total variance.

The texture attributes and flavours drive the first and the second dimensions, respectively, and this is consistent with the results reported by Echeverría et al. (2008). In our study, crispness, fibrousness, crunchiness and hardness were positively correlated to each other (mean r = 0.77), but negatively correlated to graininess and flouriness (mean r = -0.47). Sweet taste did not seem to be related to juiciness (r = 0.30), graininess or flouriness (r = 0.19 for both of them). Harker et al. (2006) hypothesised the existence of a relationship between juice release and sweetness in apples: sweet taste perception could depend on the breakdown of fruit flesh during chewing, rather than on differences in the sugar and acid contents. However, their results from the sensory evaluation by a trained panel did not support this hypothesis. They then suggested that even a small volume of juice could be sufficient to stimulate the sensory response to sugars. Echeverría et al. (2008) found an interaction between sweetness and mealiness perception, but the relationship was not supported by a correlation between the two variables: the perception of sweetness was influenced by mealiness in a way that can be explained with an anticlockwise rotation (sweetness-mealiness), with samples displaying a high degree of mealiness perceived as less sweet than the current value, while those exhibiting a low degree of mealiness were perceived as being sweeter, even if texture properties were neither linearly nor monotonically related to sweet taste perception.

It can be observed in the cultivars distribution in the GPA product map (Fig. 3) that ‘Fuji’, ‘Cripps Pink’ and ‘Granny Smith’ are characterised by higher values of crunchiness, crispness, hardness, fibrousness and juiciness (plotted on the right side of the plot); grainy and floury varieties, such as ‘Renetta Canada’, are located on the left quadrant. Along the second GPA dimension, sweet, high odour and yellow apples, such as ‘Golden Delicious’ and ‘Gala’, are discriminated from the acidic, astringent and green varieties (‘Granny Smith’, ‘Renetta Canada’) (Fig. 3). Karlsen et al. (1999) found that variance along the first component in the PCA for apple data from sensory analysis depended on a flavour-odour factor, probably due to the high number of specific odour and aroma attributes (17 in a whole lexicon of 23). In our study, flavour attributes were considered as major factors, but they turned out to be secondary if compared to the relatively high number of texture attributes, which allowed the assessors to describe the samples mainly by their textural characteristics.

### *Sensory profiling during postharvest*

The effect of postharvest storage on the sensory properties of apples was studied for 12 apple varieties (see Table 1). The GPA bi-plot (Dim.1: 48.4%; Dim.2: 22.2%) in Figure 4 shows that textural attributes contribute to the maximum variability among the apple cultivars. It is worth noting that there is a progressive shift for each variety, from the left to the right side of the graph, as storage time increases. The two extremes of this variation are represented by the hardness-crunchiness and graniness-flouriness attributes; this distribution is consistent with the structural modifications occurring in the cell wall/middle lamella as a consequence of the solubilisation and depolymerisation of pectic substances during postharvest (Billy et al., 2008; Zdunek et al., 2010b). The general trends can be observed in the univariate two-way ANOVA with cultivar, time in storage and interaction effects as variables on a subset of data based on 7 cultivars (data not shown). All the attributes allowed for the discrimination of the apple cultivars, confirming the results obtained by the one-way ANOVA described in section 3.2. Parameters, such as sweet taste, flouriness and graininess, increased with time in storage, while crunchiness, crispness, hardness, fibrousness, juiciness, sour taste and green flesh decreased ($p < 0.001$). No time effect was observed for overall flavour ($p = 0.377$), overall odour ($p = 0.147$), astringency ($p = 0.487$) and yellow flesh ($p = 0.051$). The interaction between cultivar and time was significant for all of the texture attributes, acidity and green colour ($p < 0.001$), indicating that different changes depended on the variety and on the attribute. In Figure 5, the spider plots comparing the sensory profile at different storage times are shown for four cultivars, chosen as examples of different development trends. Differences in texture parameters were observed from 1 to 2 months postharvest for ‘Renetta Canada’ (Fig. 5a) and ‘Cripps Pink’ (Fig. 5b), as they passed from an unripe condition to complete maturity. For ‘Pinova’ and ‘Granny Smith’, significant changes were observed mainly between 2 and 4 months in storage due to the polygalacturonase enzyme activity (Wakasa et al., 2006; Costa et al., 2010b) that makes the fruit reach a over-ripen condition during this postharvest phase. ‘Golden Delicious’ (Fig. 5c) showed a more progressive trend from 1 to 4 months postharvest, while ‘Fuji’ (Fig. 5d) and ‘Red Delicious’ exhibited no changes in their textural properties. Other authors have confirmed that ‘Fuji’ best maintains its texture after harvest as compared with the other cultivars, probably due to low ethylene production and to a reduced expression of a polygalacturonase gene devoted to the degradation of the cell wall (Jobling and McGlasson, 1995; Mehinagic et al., 2004; Costa et al., 2010b). The favourable texture of ‘Fuji’ and its acceptance by consumers even after 61 days was reported in a previous study (Varela et al., 2008). Moreover, Costa et al. (2012) observed an improvement in the ‘Fuji’ apple’s acoustic properties (via compression) after 2 months of cold storage as compared with that at harvest. ‘Golden Delicious’, a known apple reference cultivar (Velasco et al., 2010), showed a decrease in juiciness between 1 and 2 months of storage (Fig. 5c), agreeing with the data of Mehinagic et al. (2004). Other authors have also reported on the rapid decrease in textural properties for ‘Golden Delicious’ during the early stages of storage, while flavour properties remained unchanged (Watada et al., 1980; Billy et al., 2008). For ‘Cripps Pink’ (Fig. 5b), there was a significant reduction in hardness and crunchiness between 1 and 2 months of storage as well as a decrease in sour taste from 1 to 4 months postharvest. This observation is in agreement with the results of Drake et al. (2002) who reported a significant reduction in firmness for ‘Cripps Pink’ apples between 90 and 180 days of storage in normal atmosphere at 1°C.

Among the apple cultivars investigated, ‘Renetta Canada’ exhibited the most dramatic changes in sensory properties after 2 months of postharvest storage, with a significant reduction in hardness, crispness, crunchiness, fibrousness and sour taste, accompanied by a relevant increase in flouriness and graininess. Moreover, a significant decrease in the green flesh colour was observed (Fig. 5a).

Our results show that different apple varieties exhibit different changes, although they were all subjected to the same storage conditions. This finding is in agreement with the results of other authors who showed that the mechanical properties of different apple varieties evolve differently under different storage conditions due to varying cell wall pectin composition and genetic constitution at the loci involved in pectin degradation that lead to various levels of water loss and air volume increase (Jobling and McGlasson, 1995; Johnston et al., 2001; Billy et al., 2008; Varela et al., 2008; Costa et al., 2012).

## CONCLUSIONS

We developed and described a complete strategy for the sensory characterisation of apple with detailed procedures for judge selection and training, sensory lexicon development, sample preparation and panel performance control. This protocol that is the most complete available so far for apple sensory profiling, is intended to give a reference tool for all activities aiming at improving perceived apple quality.

Method validation is of primary importance, since it represents the sensory data quality check that would be applied before using average values for product profiling or correlation analysis. We also suggest tailored statistical methods in order to ensure the accuracy of the collected results, to highlight effectiveness of the developed sensory vocabulary and to monitor eventual difficulties in the use of specific descriptors. Univariate and multivariate analyses are proposed to verify the effectiveness of attributes to highlight the perceivable differences among samples.

The validation on a large set of cultivars (21) demonstrates the utility and applicability of the proposed tool. We identified differences in sensory profiling between the different varieties and within a single variety at different postharvest storage periods. Multivariate analysis elucidated the complex relationships among the attributes used to characterise apple sensory quality.

Further research will focus on the correlation between sensory evaluation and instrumental data to give a sensory meaning to standard and innovative physical and chemical parameters (Harker et al., 2002a; Chauvin et al., 2010). Reliable sensory data on numerous cultivars can contribute to a new scientific field, called “sensomics”, to complement and assist the genetic improvement of new apple accession characterised by superior fruit quality, oriented towards the desires of consumers.

**Table 1:** Apple varieties and respective codes used in Figs. 3 and 4. Letters “a”, “b”, “c” and “d” indicate orchard different locations as described in the "Location” column. The SSC and titratable acidity are expressed as mean values.

| Variety | Code | Location | Harvest | Postharvest months | Fruit weight (g) | SSC | Titratable acidity[a] |
|---|---|---|---|---|---|---|---|
| Braeburn | BRN_a | Maso Part | 1/10/2010 | 2, 4 | 221 | 14.3 | 10.5 |
| Braeburn | BRN_b | Giaroni | 30/09/2010 | 2 | 210 | 14.3 | 9.5 |
| Cripps Pink | PIN_a | Maso Part | 20/10/2010 | 1, 2, 4 | 199 | 14.6 | 9.1 |
| Cripps Pink | PIN_b | Giaroni | 26/10/2010 | 2 | 188 | 14.0 | 8.6 |
| Delearly | DLR_b | Giaroni | 4/08/2010 | 2 | 198 | 12.9 | 10.4 |
| Florina | FLO_d | Laimburg | 14/09/2010 | 2 | 246 | 14.6 | 11.9 |
| Fuji | FJ_a | Maso Part | 5/10/2010 | 1, 2, 4 | 247 | 15.6 | 5.7 |
| Fuji | FJ_b | Giaroni | 1/10/2010 | 2 | 268 | 17.0 | 5.4 |
| Gala | GAL_b | Giaroni | 23/08/2010 | 2 | 170 | 14.4 | 5.2 |
| Gloster | GLO_b | Giaroni | 14/09/2010 | 2 | 250 | 13.1 | 7.6 |
| Gold Rush | GDR_b | Giaroni | 30/10/2010 | 2 | 271 | 14.8 | 10.5 |
| Golden Delicious | GOL_a | Maso Part | 24/09/2010 | 1, 2, 4 | 250 | 15.2 | 9.4 |
| Golden Delicious | GOL_b | Giaroni | 16/09/2010 | 2 | 222 | 12.4 | 8.2 |
| Granny Smith | GRA_a | Maso Part | 30/09/2010 | 1, 2, 4 | 222 | 15.6 | 14.6 |
| Granny Smith | GRA_b | Giaroni | 30/09/2010 | 2 | 257 | 11.9 | 12.4 |
| Idared | IDA_b | Giaroni | 30/09/2010 | 2 | 250 | 13.2 | 8.1 |
| Modì | MOD_b | Giaroni | 7/09/2010 | 2 | 175 | 15.1 | 6.6 |
| Morgenduft | MOR_a | Maso Part | 1/10/2010 | 2, 4 | 235 | 11.5 | 9.6 |
| Pilot | PIL_b | Giaroni | 15/09/2010 | 2 | 225 | 12.5 | 9.7 |
| Pinova | PNV_c | Maso Maiano | 28/09/2010 | 1, 2, 4 | 224 | 16.6 | 9.4 |
| Red Chief | RCF_b | Giaroni | 7/09/2010 | 2 | 269 | 12.7 | 4.2 |
| Red Delicious | RED_c | Maso Maiano | 20/09/2010 | 1, 2, 4 | 223 | 10.0 | 4.1 |
| Renetta Canada | REN_c | Maso Maiano | 20/09/2010 | 1, 2, 4 | 253 | 13.9 | 9.4 |
| Renetta Canada | REN_b | Giaroni | 7/09/2010 | 2 | 319 | 14.6 | 13.8 |
| Rubens | RUB_c | Maso Maiano | 21/09/2010 | 2, 4 | 203 | 13.9 | 6.0 |
| Stayman | STY_a | Maso Part | 4/10/2010 | 2, 4 | 278 | 12.4 | 7.2 |
| Topaz | TOP_c | Maso Maiano | 28/09/2010 | 2, 4 | 237 | 14.3 | 13.3 |

[a] meq malic ac./100 g juice

**Table 2:** The tests implemented in the preliminary phase of panel training and selection: Tests 1, 3, 5, 7 and 9 concerned taste stimuli (Table 2a) and tests 2, 4, 6 and 8 concerned odour stimuli (Table 2b).

**a.**

| **Test** | **Training on TASTE: test task** | **Substances and concentration (g/kg)** |
|---|---|---|
| 1 | Recognition of 12 taste stimuli: acid, sweet, salty and bitter in water solutions. | Chemicals [a]: Caffeine (0.4). Citric acid (1.0), Saccharose (10.0), Sodium Chloride (1.5) |
| 3 | Scaling of 9 taste stimuli: acid, sweet and bitter in water solutions at 3 different intensities | Chemicals [a]: Caffeine (0.15-0.6), Citric acid (0.5-2.0), Saccharose (20.0-80.0) |
| 5 | Scaling of 12 taste stimuli: acid, sweet and bitter in water solutions at 3 different intensities | Chemicals [a]: Caffeine (0.15-0.6), Citric acid (0.5-2.0), Saccharose (20.0-80.0) |
| 7 | Recognition of 12 taste stimuli: acid, sweet and bitter in water solutions at 2 different intensities | Chemicals [a]: Caffeine (0.3-0.6), Citric acid (1.0-2.0), Saccharose (40.0-80.0) |
| 9 | Scaling of 9 taste stimuli: acid, sweet and bitter in apple juice[b] at 3 different intensities | Chemicals [a]: Caffeine (0.2-0.8), Malic acid (1.0-4.0), Fructose (10.0-40.0) |

**b.**

| **Test** | **Training on ODOUR: test task** | **Substances and concentration (mg/kg)** |
|---|---|---|
| 2 | Recognition of 12 odour stimuli: aromas adsorbed on cotton wool (in 40-ml glass vials) | Aromas[c:] Lemon; Orange; Pineapple; Banana; Melon; Apple; Pear; Strawberry; Raspberry; Cherry; Apricot; Peach (3 drops, approximately 0.15 ml/vial) |
| 4 | Recognition of 12 odour stimuli: aromas adsorbed on cotton wool (in 40-ml glass vials) | Aromas[c:] Almond; Linden; Rose; Violet; Green pepper; Mushroom; Liquorice; Cut hay; Thyme; Vanilla; Cinnamom; Clove (3 drops, approximately 0.15 ml/vial) |
| 6 | Recognition of 12 odour stimuli in a hydroalcoholic solution | Chemicals[d]: Anethole (0.5), β-Damascenone (5.0), Diacetyl (5.0), D-Limonene (10.0), Ethyl Hexanoate (5.0), Etil Acetato (50.0), Geraniol (10.0), β-Ionone (5.0), Linalool (5.0), Vanillin (10.0) |
| 8 | Recognition of 12 odour stimuli in a hydroalcoholic solution | Chemicals[d]: Acetic Acid (5000), Benzaldehyde (5.0), Butyric Acid (100), Cinnamaldehyde (5.0), Cis-3-Hexen1-ol (20.0), Citronellol (10.0), Ethyl Butanoate (1.0), n-Hexyl Acetate (5.0), Isoamylacetate (5.0), L-Mentolo (10.0), Methyl Anthranilate (0.5), Thymol (5.0) |

[a] provided by Carlo Erba Reagenti S.p.A. (Arese, MI, Italy) - food grade
[b] 100% apple juice, Pfanner Getränke GmbH, Lauterach, Austria
[c] from Nez du Vin master kit (www.lenez.com)
[d] provided by Sigma-Aldrich Co. LLC (St. Louis, MO, USA) and Carlo Erba Reagenti S.p.A. (Arese, MI, Italy)

**Table 3:** The sensory lexicon developed by the panel. For each attribute, the sensory definition, the evaluation procedure and the references are shown.

| Category | Attribute | Sensory definition | Evaluation procedure | Reference 0 | Reference 100 |
|---|---|---|---|---|---|
| Appearance | Green Flesh | The green tint of flesh | Note the colour and evaluate the green gradation in white colour | Printing of white colour (RGB model: red 255; green 255; blue 255) | Printing of green colour (RGB model: red 207; green 253; blue 203) |
| Appearance | Yellow Flesh | The yellow tint of flesh | Note the colour and evaluate the yellow gradation in white colour | Printing of white colour (RGB model: red 255; green 255; blue 255) | Printing of yellow colour (RGB model: red 252; green 237; blue 150) |
| Texture | Hardness | Resistance of the sample to the first chews with molars | Place the sample between the molars and press without breaking it (1-2 times), evaluating the resistance | Carrot boiled for 12 min. | Carrot boiled for 4 min. |
| Texture | Crispness | Sound (pitch/intensity) produced by the sample at the first bite using the fore teeth | Place the sample between the incisors, break it by a single bite and evaluate the sound | Wet breakfast cereals[a] | Dry breakfast cereals |
| Texture | Juiciness | Amount of juice released during chewing (first three chews) | Place the sample between the molars, chew 3 times quickly and create a depression to evaluate the amount of released juice | Unripe melon | Ripe melon |
| Texture | Crunchiness | Sound (pitch/intensity) produced by the sample during 5 molar chews. | Place the samples between the molars, chew 5 times and evaluate the sound | Wet breakfast cereals[a] | Dry breakfast cereals |
| Texture | Flouriness | Degree of breaking in small and dry fragments/granules during chewing. | Chew the mouthful until it is ready to be swallowed and evaluate the tendency to make a small, soft and dry mass | Potato boiled for 4 min. | Potato boiled for 12 min. |
| Texture | Fibrousness | Degree of flesh breaking during chewing in thick and fibrous fragments/granules, until the mouthful is ready to be swallowed | Place the sample between the molars, chew until the mouthful is ready to be swallowed and evaluate the presence of fibres (perceivable as thick flesh fragments) | Carrot boiled for 12 min. | Raw celery |

| | | | | | |
|---|---|---|---|---|---|
| Texture | Graininess | Numbers/size of fragments/granules produced during chewing | Place the sample between the molars, chew 5 times and evaluate amount and size of the fragments | Carrot boiled for 4 min. | Shortbread biscuit |
| Odour | Overall odour | Overall odour sensation (perceived by smelling) | Open the lid of the cup, smell and quantify the intensity of all perceived odours | Apple juice[b] diluted 1:2 | Apple juice[b] as it is |
| Flavour | Sweet taste | Sweet taste sensation | Evaluate the intensity of sweet taste | Fructose water solution 20 g/Kg | Fructose water solution 80 g/Kg |
| Flavour | Sour taste | Sour taste sensation | Evaluate the intensity of sour taste | Citric acid water solution 0.6 g/Kg | Citric acid water solution 2.0 g/Kg |
| Flavour | Bitter taste | Bitter taste sensation | Evaluate the intensity of bitter taste | Caffeine water solution 0.15 g/Kg | Caffeine water solution 0.6 g/Kg |
| Flavour | Astringency | Tactile sensation of dryness in the mouth | Chew until the mouthful is ready to be swallowed and evaluate the intensity of dryness/friction sensation (tongue and mucosa) after swallowing | Tannic acid water solution 0.1 g/Kg | Tannic acid water solution 0.5 g/Kg |
| Flavour | Overall flavour | Overall flavour sensation by retro-nasal evaluation (through the mouth to the nose) | Chew until the mouthful is ready, swallow and quantify the intensity of all the odour stimuli perceived retro-nasally | Apple juice[b] diluted 1:2 | Apple juice[b] as it is |

[a] 50 g breakfast honey balls extruded cereals (Miel Pops Kellogg’s) were kept for 24 h at 23°C in a sealed bin together with a cup of 30 ml water.

[b] 100% cloudy apple juice produced by Pfanner Getränke GmbH, Lauterach, Austria.

**Fig. 1:** *p*-MSE plot. The dashed area highlights the ideal combination for a good assessor ($p < 0.05$; MSE < 400). Outside this area, “problematic” judges (different symbols indicate different assessors) and “problematic” attributes are reported.

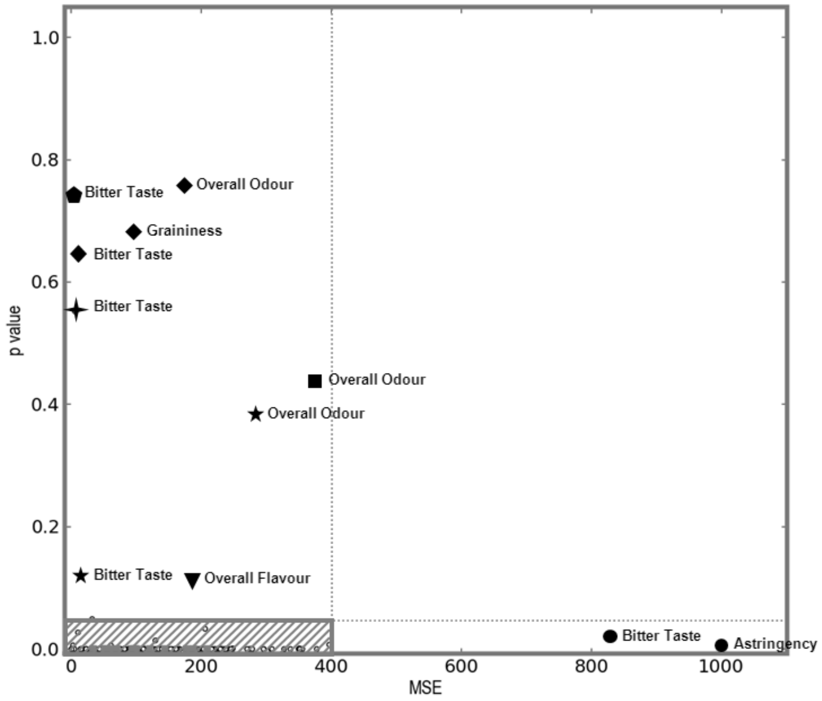


**Fig. 2:** Tucker-1 plots for “hardness” (A) and “bitter taste” (B) attributes. The external ellipse represents 100% of the explained variance; the inner ellipse represents 50% of the explained variance. The position of each assessor (indicated by their personal codes) provides information about his/her agreement to the panel average.

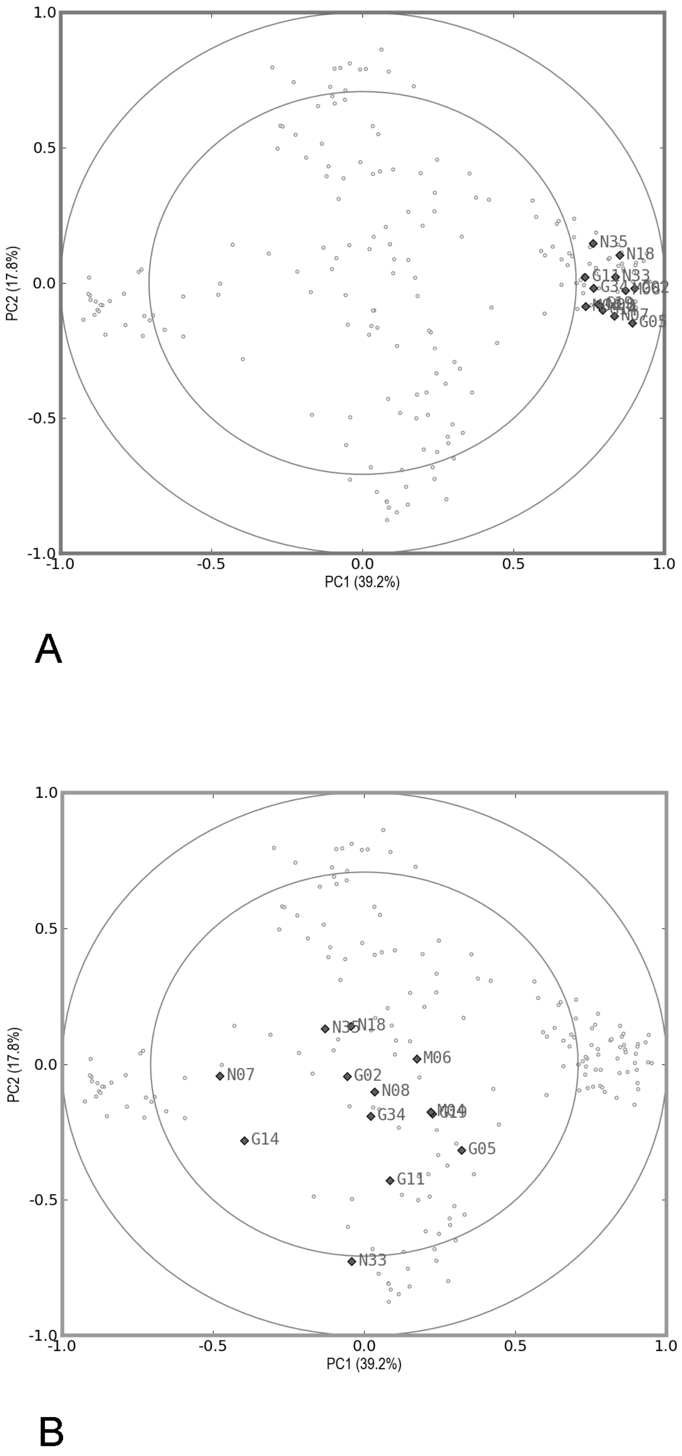


**Fig. 3:** GPA bi-plot (Dim.1: 47.6%; Dim.2: 18.8%) showing the profiles of the 21 apple varieties analysed two months postharvest. Letters “a”, “b”, “c” and “d” following the sample codes refer to the origin orchard locations (see Table 1).

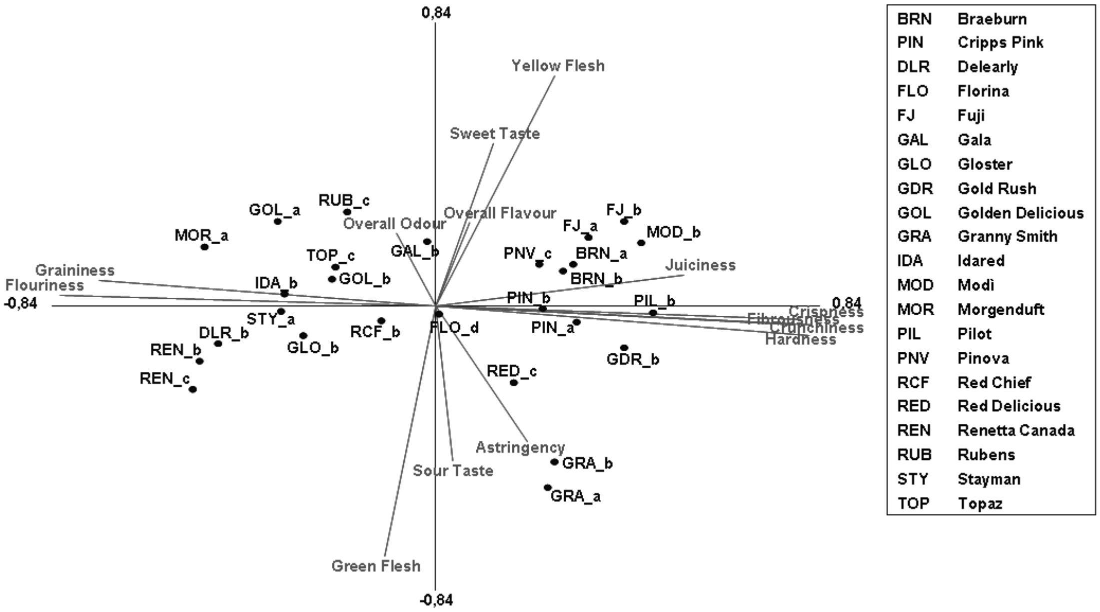


**Fig. 4:** GPA bi-plot (Dim.1: 48.4%; Dim.2: 22.2%) showing the profiles of 12 apple varieties analysed at different times during storage. Letters “a”, “b”, “c” and “d” following the sample codes refer to the origin orchard locations; the number specified for each sample indicates the storage period before the analysis: 1, 2 or 4 months.

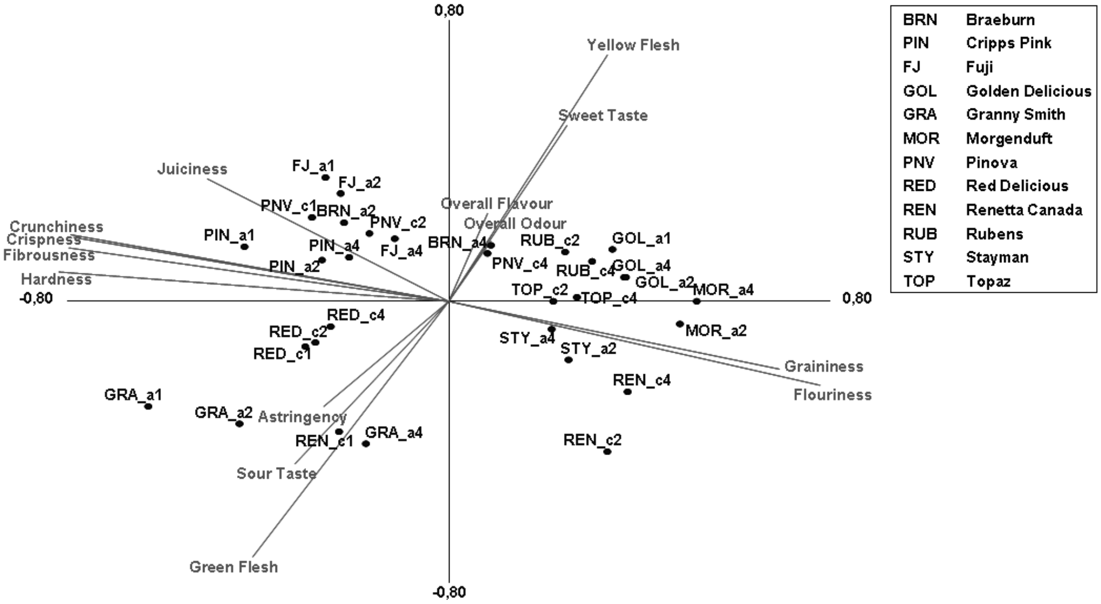
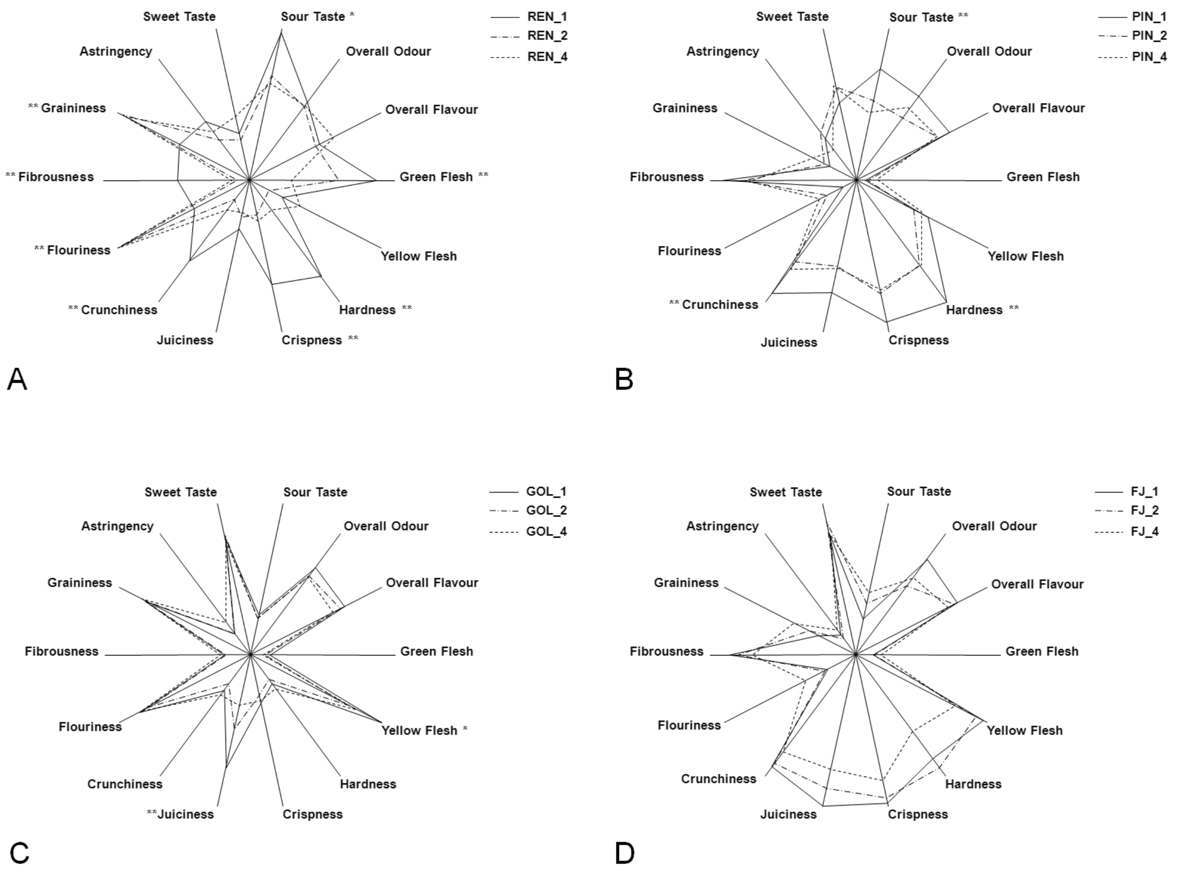


**Fig. 5:** Spider plots showing the sensory profiles of four varieties (‘Renetta Canada’, A; ‘Cripps Pink’, B; ‘Golden Delicious’, C; ‘Fuji’, D) analysed at three different storage periods. The numbers following the cultivars code in the legend represent the storage period (1, 2 and 4 months).



# *Chapter III*

# A COMBINED SENSORY-INSTRUMENTAL TOOL FOR APPLE QUALITY EVALUATION

## INTRODUCTION

Texture properties of fruit and vegetables are considered the most important drivers of consumer choice, followed by flavour characteristics (Daillant-Spinnler et al., 1996; Jaeger et al., 1998; Péneau et al., 2006 and 2007; Harker et al., 2008). Food suppliers currently measure apple quality considering basic pomological descriptors, such as fruit shape, size, colour, soluble solids content, titratable acidity, and penetrometry measurements, that is the most frequently used method for measuring fruit mechanical properties (Harker et al., 1997; Hoehn et al., 2003). Sensory analysis is not often considered because it is expensive and has a constrained sample set due to the limitations in employing human beings as instrument. Moreover, it cannot be used for measuring quality properties in real time. This is an issue particularly for agricultural products since their high variability requires wide samplings. Additionally, the quality assessment of breeding materials, normally represented by a single plant/individual, can count on a restricted availability of samples, which could be not sufficient for sensory panel evaluations. However, the best way to precisely describe the eating quality of food is the sensory approach, which is able to define, measure, quantify, and explain what is really perceivable by the human senses

(Carbonell et al., 2008). Descriptive sensory analysis, in particular, provides a comprehensive qualitative and quantitative sensory description of a product (Murray et al., 2001). To overcome sensory methods' limitations and to allow the quality characterisation on a large variety of materials, the estimation of sensory attributes by instrumental measures would represent a valid opportunity. The majority of these investigations are addressed to texture properties (De Belie et al., 2002; Harker et al., 2002a; Mehinagic et al., 2003; Chauvin et al., 2010). Harker et al. (2002a) studied various instrumental measures to predict texture sensory attributes, showing a possibility to predict sensory firmness, crispness, crunchiness, initial juiciness, and ease of breakdown through a puncture test, and that a minimum difference of 6-8 N in instrumental firmness is necessary to have a difference in perceived sensory attributes by a trained sensory panel (Harker et al., 2002a). Chauvin et al. (2010) found a strong correlation between texture sensory attributes and compression measurement by texture analyser. Mehinagic et al. (2003) compared the use of measures by penetrometry with non-destructive vis/NIR analyses, looking at correlations with sensory assessments, in order to propose non-destructive measurements as an alternative. Brookfield et al. (2011) proposed the use of small panels (< 4 subjects) as a cheaper alternative to measure apple texture. They concluded that the panel works well if focused on a very small number of attributes (such as crispness and juiciness). The same authors highlighted that the instrumental-sensory relationship did not follow a unique trend, because each cultivar tends to respond in different ways to the different tests (Brookfield et al., 2011). This observation suggests that a wide set of apple varieties, representing a wide range of variability for several sensory apple attribute, should be considered in such studies. Human assessment should always be considered a reference to calibrated instrument readings, in order to develop tools falling within the range of textural parameters known to be accepted by consumers (Bourne, 2002; Harker et al., 2003).

While perceived texture can sometimes be predicted by instrumental data, the case of flavour and taste attributes is, in general, more difficult. Many studies, for instance, underline the difficulties in developing a model to predict sweet taste, eventually finding conflicting results about the relation between sweetness and texture properties (Harker et al., 2002b; Harker et al., 2006; Echeverría et al., 2008). It is therefore important to consider predictions of any sensory attribute could hold a potential influence on other properties not directly related to it. This is particularly true in the case of flavours, which derives from an integration of different information coming from several senses (taste, smell and tactile stimuli; see Prescott, 2012; Small, 2012).

In 2011, Costa et al. proposed the use of a texture analyser which was employed to dissect apple fruit texture in several components, by simultaneously profiling the mechanical and the acoustic components. The method was previously applied on a wide set of 86 different apple varieties. The data acquired were compared with the sensory texture profiles provided by a restricted panel of apple experts, who evaluated a sub-set of 21 apple varieties for firmness, crispness, and juiciness attributes. Regression analyses highlighted that the instrumental force parameters from texture analyser measurements were necessary to predict both firmness and crispness sensory attributes, and that a high correlation between acoustic parameters and the sensory attribute of crispness does exist (Costa et al., 2011).

Here, we propose a complete methodology for sensory profiling of apples. This was applied in parallel to instrumental measures of some physical and chemical properties, including texture analyser measurements as proposed by Costa et al. (2011), dry matter concentration, extractable juice content, colorimeter measurements, and basic chemical composition, on a wide set of apple varieties along a two-year period, in order to study the sensory profiles of cultivars having the highest possible variability in their sensory properties.

## MATERIALS AND METHODS

### *Plant materials*

A set of 27 commercial apple varieties (*Malus* × *domestica* Borkh.) was analysed over two seasons (2010 and 2011), with 18 common cultivars shared between both years of experiment. Six varieties in season 2010 and two in season 2011 were evaluated twice, since they were harvested from different orchards managed by Fondazione Edmund Mach (FEM; San Michele all'Adige, Trento, Italy) and Laimburg Research Centre for Agriculture and Forestry (Laimburg, Bolzano, Italy) (Table 1). In 2011, two additional clones were analysed for two varieties: Roho 3615 for Pinova variety and Red Spur Jeromine for Red Delicious. All orchards were managed according to standard agronomical practices (i.e. thinning, pruning, and pest control). The fruits were picked at commercial harvest, determined by the standard descriptors used to monitor fruit maturity and ripening, such as flesh firmness, skin colour, total acids, sugar content, and starch degradation index. For each sample, a minimum of 20 apples of homogeneous size and without any visible external damage were selected and stored for two months in normal atmosphere at

2°C and 95% relative humidity. Prior to the analyses, fruit were kept at room temperature for 24 hours.

Samples were prepared in accordance to Corollaro et al. (2013), cutting peeled flesh cylinders (1.8 cm diameter; 1.2 cm height) from three slices cut around the equatorial plane perpendicular to the core of the fruit. Thus, every cylinder had the main axis being parallel to the fruit core. The cylinders were immediately treated with an antioxidant solution (0.2% citric acid, 0.2% ascorbic acid, 0.5% calcium chloride). Cylinders coming from the same fruit were used for both sensory (8 cylinders put into clear plastic cups encoded with a random three-digit code) and instrumental analyses. Sensory evaluations were performed within one hour from fruit cutting; instrumental analyses within three hours. All the measurements were performed after the antioxidant treatment, to ensure that sensory and instrumental data were reliably comparable.

### *Sensory analysis*

The sensory panel included 13 judges in 2010 (6 males; 7 females) and 14 in 2011 (4 males; 10 females), all FEM employees, with seven common judges for both years. Panellists were trained as reported in Corollaro et al. (2013). Sensory profiling was performed based on the quantitative descriptive method (Stone and Sidel, 2004a). The sensory lexicon was developed using the consensus method (Murray et al., 2001). In 2010, it was composed of attributes related to flesh colour, odour, texture, and flavour. Univocal definition, evaluation procedure, and reference standards for each attribute are reported in Corollaro et al. (2013). Odours (ortho-nasal perceptions by smelling) and flavours (retro-nasal perceptions by tasting) were evaluated both by the overall intensity and by a set of 31 specific attributes (Aprea et al., 2012). In 2011, the lexicon was the same as 2010, with the exception of “bitter taste”, which was removed as it was not discriminant, and “crispness”, which was redundant due to its strong positive correlation to crunchiness ($r = 0.99$; $p < 0.001$). The specific sensory attributes for odour and retro-nasal flavours were reduced to nine.

In this work, only the 11 attributes related to appearance, texture, and flavour common to both seasons were considered (Table 2), while the profiles related to specific odour and flavour attributes were preliminary investigated in Aprea et al. (2012).

The intensity of each attribute was scored by the panel on a 100 mm linear scale, anchored at 0 (absence) and 100 (extremely intense), with an anchor at halfway (50). The sensory tests were performed once per week in individual computerised booths equipped with FIZZ software (2.46A, Biosystemes, Couternon, France) under white artificial lighting. Unsalted bread and water were provided to the assessors to cleanse their palate between samples. Six apple samples were analysed per session, according to a randomised balanced order over the assessors and two replicates performed for each sample (three varieties in duplicate per session). The sessions took place once a week (in a few cases, twice a week) from October to December, both in 2010 and 2011.

### ***Instrumental analyses***

#### *Colour analysis*

L*a*b components from CIELAB colour space model (see Schanda, 2007) were measured on four samples of flesh cut from each fruit using a CR-400 colorimeter, supported by the CM-S100w SpectraMagic™ colour data software (Konica Minolta Sensing, Inc., Japan).

#### *Texture analysis*

Texture properties were measured on flesh cylinders (ten cylinders sampled from ten different fruit per each variety; each cylinder was considered a replicate of that variety) by a TA-XT texture analyser equipped with an acoustic envelop detector device (Stable MicroSystem Ltd., Godalming, UK), using a 4 mm probe to compress the samples. Twelve mechanical and four acoustic parameters were calculated on the recorded curves, following the method described by Costa et al. (2011; Table 3).

#### *Juice extraction and dry matter concentration*

The extractable juice was measured by weighing the liquid expressed from the mechanical compression of eight flesh cylinders per variety (each cylinder coming from a different fruit) and expressed as percentage of fresh weight. Dry matter concentration was measured by drying eight flesh cylinders per variety at 105°C until stable weight.

### *Basic chemical measurements*

The juices squeezed from each cultivar (12 cylinders sampled from different fruit) was measured for % of soluble solids concentration (SSC) with a DBR35 refractometer (XS Instruments, Poncarale, Brescia, Italy) and titratable acidity with a Compact Titrator (Crison Instruments S.A., Alella, Barcelona, Spain), both in two replicates (Table 1). NaOH 0.1N was used to titrate the samples to pH 8.16. The results were calculated as mail acid milliequivalents in 100g juice.

### ***Statistical analysis***

The averaged sensory and instrumental profiles were evaluated using univariate and multivariate approaches, applied to the complete data-set including both apple seasons, except for specific analyses applied on only 2011 data, as indicated below.

One-way ANOVA on instrumental data and Pearson's correlations between sensory attributes and between instrumental parameters were performed by STATISTICA 9.1 software (StatSoft Inc., U.S.A.). To visualise the sensory space, a Principal Component Analysis (PCA) was performed on the sensory data-set through the same software. The Unscrambler v9.8 software (CAMO Software, Norway) was used to study the relation between sensory and instrumental data through Partial Least Square (PLS-2 and PLS-1) regression analyses. Box-Cox transformation (Box and Cox, 1964) of the instrumental data was evaluated by STATISTICA 9.1 software before PLS analyses.

### *Panel performance evaluation*

Panel performances were evaluated on both the 2010 and 2011 data-sets to validate the sensory method through a three-way mixed ANOVA (considering judge as random factor, and product and replicate as fixed factors). Main effects were studied, in which a *p*-value lower than 0.05 indicated significant differences. In both seasons, the judge effects were significant for every attribute, as expected in sensory data, since each judge contributes differently to describe the variability between the samples. However, the existence of a judge effect did not influence the product effect which was significant in both seasons, demonstrating that the two panels were able to discriminate between different apple varieties with good reproducibility. The replicate effect was significant for only three attributes in the 2011 data-set, showing a slightly lower reproducibility in the 2011 panel. However, problems related to specific judges and/or specific attributes did not affect the overall sensory data reliability.

## RESULTS AND DISCUSSION

### ***Apple profiling***

A PCA was performed on the sensory data describing the apple samples from the two-year data-set. The first two principal components explained 78% of total variance in the sample set. In Figures 1a and b, the loading and the score plots are shown, respectively.

In Figure 1a, the first principal component is led by texture attributes, while the second one is related to external appearance and flavour properties, confirming texture was responsible for most of the variance existing among the samples, in agreement with other authors (Mehinagic et al. 2003; Echeverría et al., 2008). The distribution of the scores in Figure 1b shows that the same varieties analysed in the two consecutive seasons were described in a consistent manner by the trained panel, being close to each other on the plot. This result confirms that the sensory protocol is effective in providing a reliable description of the apples sensory profile: Fruit from the same variety showed sensory profiles that are maintaned from one year to another. Floury and acid varieties are located in the right-down quadrant (‘Canada Reinette’, ‘Gloster’); grainy and sweet varieties are in the right-up part of the plot (‘Golden Delicious’, ‘Gala’, ‘Morgenduft’, Rubens); hard, crunchy, and sour apples in the left-down side (‘Granny Smith’, Goldrush); crunchy and sweet varieties in the left-up quadrant (‘Fuji’, ‘Pinova’, ‘Modì’).

The directions of the loadings visible in Figure 1a is a good representation of the correlations between the attributes measured by Pearson’s correlation coefficients, showing that crunchiness, hardness, and fibrousness were negatively correlated to graininess and flouriness ($r < -0.86$; $p < 0.001$). Sweet taste was only slightly correlated to juiciness, for $r = 0.43$, $p = 0.01$. No correlation between sweet taste and flouriness or graininess was found. Actually, the relationship between juiciness/mealiness and sweetness has been deeply investigated in the currently available literature, starting from the hypothesis that sweetness perception is influenced by texture properties (thus, it could depend directly on juiciness or mealiness intensity). Echeverría et al. (2008), in particular, highlighted a relationship between sweetness and mealiness which was clear only after applying a nonnegligible rotation factor in their Generalised Procrustes Analysis. The rotation made high mealiness values match with low sweetness values. This effect was not supported by a linear correlation between the two factors (r = -0.15). Harker et al. (2006) supposed that sweetness perception could depend on the degree of breakdown of apple flesh during chewing, rather than on differences in sugar and acid content. Therefore, the authors suggested the existence of a relationship between juice release and sweetness perception. However, their results do not support this hypothesis in a clear way. Moreover, Echeverría et al. (2008) highlighted a low consensus in their sensory panel for sweetness attribute. We confirm this finding, with a poor agreement in our sensory panels on the use of sweet taste attribute (Pearson's correlation coefficient r = 0.54 and 0.57 in 2010 and 2011, respectively, with an average correlation between the panellists higher than 0.7 in both seasons). The supposed interference by other sensory properties on sweetness perception could be proposed as an explanation for that, even if no clear evidence of such relation does exist in our results.

As for instrumental measurements, one-way ANOVA on instrumental data shows significant differences between the varieties for all the performed instrumental measurements. In the case of apple texture, all 16 mechanical and acoustic parameters proposed in the method developed by Costa et al. (2011) gave significant differences, confirming their effectiveness to discriminate apple cultivars based on their different texture profiles.

The correlation analysis between the different instrumental parameters showed that the mechanical parameters coming from texture analyser measurements were correlated to the acoustic parameters, with r ranging between 0.42 ($p < 0.05$) and

0.91 ($p < 0.001$), confirming the results reported by Costa et al. (2011), who found correlations of 0.50-0.76 between mechanical and acoustic parameters. This result demonstrates the strict relation between structural properties and acoustic response in the apple flesh (Vincent, 1998). The acoustic parameter AUX1 also showed a slight positive correlation with the % of extractable juice, with r = 0.52 ($p = 0.004$). Indeed, the typical "crispy" sound is due to a high internal turgor pressure and to the integrity of the cell wall structure. Upon compression, the breakdown of this polysaccharide architecture releases the pressure together with the internal compartmented liquid content (Duizer et al., 2001). The SSC resulted positively correlated with the % of dry matter (r = 0.51, $p = 0.05$) which, according to the literature (McGlone et al., 2003; Palmer et al., 2010), is the result of the starch solubilisation process occurring during ripening.

### ***Sensory-instrumental relationship***

In order to have an overview about the relation between sensory and instrumental parameters, the whole sensory and instrumental data-set was subjected to PLS-2 analysis. In Figure 2, the x and y loadings are shown, with both instrumental mechanical-acoustic and sensory texture properties defining the first principal component, and chemical and sensory taste properties outlining the second one. This result confirmed that most of the variability observed among the apple varieties is due to texture properties, as seen in the sensory profile discussed in "Apple profiling" paragraph. Sensory texture attributes were strictly related to mechanical and acoustic texture parameters. Juiciness, instead, was less correlated to the texture analyser data, but strongly related to the % of extractable juice. Sour taste attribute was highly related to titratable acidity. Sweet taste could not be linked to SSC. L*a*b parameters, acquired with the digital colorimeter, were related to flesh colour sensory attributes. As expected, yellow flesh intensity was positively related to the b* measure, which increases as the light wavelength passes from blue to yellow range (Schanda, 2007). Interestingly, sweet taste attribute appeared to be also related to the colorimeter data (Fig. 2).

Such observations were the starting point for the development of predictive models for each sensory attribute.

### *Predictive models*

The sensory and instrumental data-set was subjected to PLS-1 analyses, in order to find the best prediction model for each sensory attribute. In Table 4, PLS-1 models and validated $R^2$ for each sensory attribute using different series of instrumental data are reported. Before performing the analyses, Box-Cox transformation of the instrumental data was evaluated: In Table 4, the indication about the use of transformed or untransformed data is indicated. The use of transformed data was a critical key-point for the prediction of taste sensory attributes.

For appearance and texture attributes, the prediction well fitted untransformed data, because of their normalised distribution. For each sensory attribute, a model using instrumental parameters corresponding to its specific sensory description was first developed, e.g. colorimeter data for flesh appearance attributes. However, better models were usually achieved using a combination of different instrumental variables which are indirectly related to the sensory attribute. Thus in Table 4, only the best prediction model for each attribute is reported. The models using chemical and colorimeter data ("Colour + Chemical", as indicated in Table 4) were developed based on the 2011 data-set, because colorimeter measurements were included in the instrumental protocol only in the second season of activity.

### *Appearance attributes*

An effective prediction of flesh colour (green and yellow) was achieved in 2011, after the addition of colorimetric measurements to the set of instrumental analyses. A better result was obtained for yellow flesh compared to green flesh. Interestingly, in both cases the best models were achieved using chemical parameters (i.e., SSC and titratable acidity) rather than colorimetric data alone (Table 4). Indeed, flesh colour tends to go from green to yellow as the fruit passes from an unripe to ripe condition, as the pigment content changes from having a high concentration of chlorophyll to having a high concentration of carotenoids (Ampomah-Dwamena et al., 2012). The ripening mechanism also involves chemical compounds, with a reduction of acid content and an increase in SSC/titratable acidity ratio (Jan and Rab, 2012). Thus, a combination of colorimetric and chemical data provides better information to predict flesh colour.

### *Texture attributes*

The different parameters defined to assess the fruit texture by texture analyser measurements were adequate to efficiently predict all the texture sensory attributes (with $R^2 \geq 0.77$) with the exception of juiciness. The best model for juiciness attribute was achieved by using the whole instrumental data-set. Significant variables in the prediction model were texture analyser data, % of extractable juice, L* parameter from colorimeter analyses, and titratable acidity, suggesting that a relation between tastants and juiciness perception may exist. The relation highlighted by PLS-1 analysis between juiciness and titratable acidity is negative: the higher the acid concentrations, the lower the juiciness score. This negative correlation was already observed in the PLS-2 analysis between juiciness and titratable acidity (Fig. 2; see “Sensory-instrumental relationship” paragraph). Mechanical parameters from the texture analyser appeared to have different contributions for the prediction of different sensory texture attributes. In general, each parameter contributed significantly to at least one predictive model. Other authors found good correlations between puncture test and sensory texture attributes evaluated by a trained panel (Harker et al., 2002a; Chauvin et al., 2010; Guerra et al., 2010). Our results confirmed that the proposed texture analyser test is effective to collect information about mechanical and acoustic properties expressed by apple tissues when consumers bite into them. Moreover, our data-sets comprise of acoustic information that were not considered in many of the previous studies. Zdunek et al. (2010a) developed a similar tool for apple texture analysis, using a contact acoustic emission detector, related to a penetrometric equipment, to record the acoustic response of apples during compression. In their work a strong positive correlation was found between crispness, crunchiness, and acoustic parameters (number of acoustic events and mean acoustic event amplitude), with a Pearson’s correlation coefficient varying from 0.6 to 0.9. However, in their investigation the variability observed was due to the fact that different fruit from the same batch for sensory and instrumental evaluations were used (Zdunek et al., 2010a). A similar limitation was also observed in the work presented by De Belie et al. (2002), which compared sensory crispness with the recorded sound produced by Royal Gala apples during biting. The authors underlined that instrumental recordings were made on a subject chewing an apple piece, while sensory scores were provided by other different volunteers of a trained sensory panel on different pieces from the same apples. The best correlation they reported was r = 0.65, because of differences in oral cavity shape and force-deformation patterns operated by the front teeth of the different subjects (De Belie et al., 2002). Also Ioannides et al. (2009) provided similar results, by the use of an electromyography of masticatory muscles on subjects evaluating texture attributes of apples. In their work, the main source of variability in the data was attributed to the subjects. Moreover, the authors found another source of variation of psychological origin in which subjects tended to chew differently when asked to score specific sensory attributes (Ioannides et al., 2009).

The advantage of our texture method, compared to the other studies discussed here, is the possibility to process samples from the same single apple, with equal shape and size, available to both sensory and instrumental measurements. The flesh cylinders cut from the same fruit were used for sensory and instrumental measurements, in order to truly compare these two data types. Moreover, the texture measurements guarantee the standardisation of the compression method, due to a specified probe speed and percentage of strain during the test. With these settings, the different acoustic responses can only refer to the actual differences between the samples.

In our work, the acoustic parameters coming from the texture analyser turned out to be significant variables used in the PLS-1 model for the prediction of crunchiness, but also for the other texture sensory attributes. This could suggest that the sensory perception of hardness, flouriness, fibrousness, graininess, and juiciness of apples is not only related to tactile and mechanical properties of the apple flesh but is also influenced by acoustic information, as seen for hardness perception in the study by Demattè et al. (submitted). Nevertheless, from our results we do not have any clear evidence of a multi-sensorial perception of texture. The reason for the apparent relation observed in the PLS-1 models could be referred to the correlation between mechanical and acoustic properties. The sound emission, related to the expansion of the cell liquid content, is possible only if strong linkages in the middle lamella exist, so that the cell walls break rather than slide against each other (Longhi et al., 2013a). This means that sound emission is only possible when the fruit flesh is characterised by specific mechanical properties, which are therefore important for the acoustic perception during biting and chewing (Vincent, 1998; Duizer, 2001). This relation was also observed for crunchiness prediction in which the model based on the 16 mechanical and acoustic parameters worked better than the prediction model based only on the four acoustic variables, increasing from $R^2$ = 0.69 to 0.85 (Fig. 3).

### *Flavour attributes*

As already observed in the PLS-2 plot discussed in “Sensory-instrumental relationship” paragraph, sweet taste attribute showed a relation with colorimetric data. The best predictions for taste attributes were obtained using a model based on chemical and colorimetric parameters, available for only the 2011 data-set , giving an $R^2$ value of 0.82 and 0.89 for sweet taste and sour taste, respectively (Table 4). This suggests a relationship between the flesh colour and the acidity or sweetness perception, which could be explained by a multi-sensorial interaction related to our previous experiences: Due to the changes in the chemical composition during ripening, it is easy to suppose an apple showing a green flesh will be sour, and vice versa. Nevertheless, different apple varieties show different flesh colours depending on genetic characteristics. Therefore, a difference in flesh colour might be indirectly related to acid or sweet taste expectations, irrespective of the real maturity stage of the fruit.

By considering these observations, we can suggest another point of view about the difficulties met by most of the authors in predicting sweetness by instrumental measures (Plotto et al., 1999; Harker et al., 2002b; Oraguzie et al., 2009). Some authors suggested a relationship between sweet perception and texture properties could exist. In our study, we could not completely explain the variability in sweet taste perception using texture data. Instead, chemical content and flesh colour properties gave a very good prediction, with a $R^2 = 0.82$ (Table 4), since sweetness perception appears to be unconsciously affected by apple flesh colour, even when sweetness is evaluated by a trained panel. This is why colorimetric data can be a valid source of information to improve the prediction of sweetness perception.

As for the astringency attribute, with the data available here, it was not possible to define a reliable prediction model, mainly because astringency is a sensation related to proanthocyanidin (PA) content (Dixon et al., 2005; Pfeiffer et al., 2006), which was not measured in our study. However, with the collected data, it seems astringency could be at least partially predicted using the complete instrumental data-set (Table 4), probably because of the link between PA content and ripening stage. As an apple ripens, the PA concentration tends to decrease (Henry-Kirk et al., 2012).

All the texture and chemical parameters evolve during ripening, so that the other instrumental parameters measured in this study can be related, even if indirectly, to the astringency intensity.

## CONCLUSIONS

Our combined sensory-instrumental approach allowed the description of a large sampling of commercial apple varieties in an effective manner, objectively defining their sensory profile and highlighting relationships among the measured physical, chemical, and sensory properties. Moreover, the performed instrumental analyses appeared to be well related to the sensory attributes, demonstrating potential relations to be studied, such as the one between colorimetric data and sweetness perception. Finally, effective predictive models were developed for: a) flesh appearance sensory properties, using colorimetric measurements; b) texture attributes, thanks to the innovative texture analyser protocol; c) and lastly taste properties, through a combination of chemical and colorimetric data. The study was carried out over two consecutive apple seasons with good results and comparable sensory descriptions of the same varieties analysed during both years, confirming that the method was correctly implemented.

The proposed combined sensory-instrumental tool can be suggested as a valid source to define sensory properties of apples in wider samplings, when sensory analysis is not feasible because of the limits in using humans, or because of a low availability of fruit material: in such cases, sensory analysis applied on a subset might allow the definition of proper predictive models to be applied on a higher number of apple samples assessed instrumentally, in order to estimate their sensory profile.

**Table 1:** Apple varieties analysed during 2010 and 2011 seasons. In “Code” column, the coding used in Figs. 1, 2, and 3 are reported: the numbers “0” and “1” following the codes refer to 2010 and 2011 respectively, for those variety analysed in both years. The letters “a”, “b”, “c”, and “d” refer to the different orchards which the samples come from, as reported in column “Location”.

| **Variety** | **Code** | **Year** | **Location** | **Harvest** | **Analysis** | **Fruit weight**[a] | **SSC**[b] | **Titratable acidity**[c] |
|---|---|---|---|---|---|---|---|---|
| Braeburn | BRN_0a | 2010 | Giaroni | 30/09/2010 | 26/11/2010 | 210.4 | 14.3 | 9.5 |
| Braeburn | BRN_0b | 2010 | Maso Part | 01/10/2010 | 30/11/2010 | 238.6 | 14.3 | 10.5 |
| Braeburn | BRN_1 | 2011 | Maso Part | 27/09/2011 | 07/12/2011 | 252.0 | 11.9 | 7.4 |
| Crimson Crisp | CRI | 2011 | Maso Maiano | 18/08/2011 | 19/10/2011 | 223.5 | 11.6 | 7.0 |
| Cripps Pink | PIN_0a | 2010 | Giaroni | 20/10/2010 | 22/12/2010 | 201.3 | 14.6 | 9.1 |
| Cripps Pink | PIN_0b | 2010 | Maso Part | 26/10/2010 | 22/12/2010 | 188.0 | 14.0 | 8.6 |
| Cripps Pink | PIN_1 | 2011 | Maso Part | 24/10/2011 | 21/12/2011 | 209.3 | 14.4 | 5.9 |
| Dalinette | DAL | 2011 | Maso Part | 11/10/2011 | 14/12/2011 | 224.1 | 15.2 | 6.7 |
| Delblush | DLB | 2011 | Maso Part | 22/09/2011 | 25/11/2011 | 261.5 | 14.1 | 6.8 |
| Delearly | DEL | 2010 | Giaroni | 04/08/2010 | 06/10/2010 | 166.1 | 12.9 | 10.4 |
| Florina | FLO | 2010 | Laimburg | 14/09/2010 | 10/11/2010 | 246.3 | 14.6 | 11.9 |
| Fuji (Kiku 8) | FUJ_0a | 2010 | Giaroni | 01/10/2010 | 30/11/2010 | 267.8 | 17.0 | 5.4 |
| Fuji (Kiku 8) | FUJ_0b | 2010 | Maso Part | 05/10/2010 | 07/12/2010 | 270.9 | 15.6 | 5.7 |
| Fuji (Kiku 8) | FUJ_1 | 2011 | Maso Part | 06/10/2011 | 07/12/2011 | 270.0 | 13.7 | 3.5 |
| Gala (Schniga) | GAL_0 | 2010 | Giaroni | 23/08/2010 | 20/10/2010 | 169.6 | 14.4 | 5.2 |
| Gala (Schniga) | GAL_1 | 2011 | Maso Part | 09/08/2011 | 12/10/2011 | 185.7 | 10.9 | 4.4 |
| Gloster | GLO_0 | 2010 | Giaroni | 14/09/2010 | 10/11/2010 | 249.6 | 13.1 | 7.6 |
| Gloster | GLO_1 | 2011 | Maso Part | 08/09/2011 | 09/11/2011 | 257.2 | 11.7 | 6.5 |
| Goldrush | GDR_0 | 2010 | Giaroni | 30/10/2010 | 22/12/2010 | 270.9 | 14.8 | 10.5 |
| Goldrush | GDR_1 | 2011 | Maso Part | 24/10/2011 | 16/12/2011 | 280.7 | 14.5 | 8.8 |
| Golden Delicious (B) | GOL_0a | 2010 | Giaroni | 16/09/2010 | 17/11/2010 | 222.1 | 12.4 | 8.2 |
| Golden Delicious (B) | GOL_0b | 2010 | Maso Part | 24/09/2010 | 24/11/2010 | 248.4 | 15.2 | 9.4 |
| Golden Delicious (B) | GOL_1 | 2011 | Maso Part | 12/09/2011 | 11/11/2011 | 255.1 | 11.8 | 3.9 |
| Granny Smith | GRA_0a | 2010 | Giaroni | 30/09/2010 | 26/11/2010 | 226.7 | 15.6 | 14.6 |
| Granny Smith | GRA_0b | 2010 | Maso Part | 30/09/2010 | 30/11/2010 | 257.4 | 11.9 | 12.4 |
| Granny Smith | GRA_1 | 2011 | Maso Part | 22/09/2011 | 25/11/2011 | 268.1 | 11.7 | 10.8 |
| Idared | IDA | 2010 | Giaroni | 30/09/2010 | 26/11/2010 | 250.4 | 13.2 | 8.1 |
| Jazz | JAZ | 2011 | Laimburg | 27/09/2011 | 30/11/2011 | 213.8 | 11.5 | 6.3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Kanzi | KAN | 2011 | Laimburg | 16/09/2011 | 23/11/2011 | 216.4 | 11.6 | 5.4 |
| Modì | MOD_0 | 2010 | Giaroni | 07/09/2010 | 03/11/2010 | 174.5 | 15.1 | 6.6 |
| Modì | MOD_1 | 2011 | Maso Part | 01/09/2011 | 02/11/2011 | 226.5 | 13.3 | 5.2 |
| Morgenduft (Dallago) | MOR_0 | 2010 | Maso Part | 01/10/2010 | 07/12/2010 | 264.7 | 11.5 | 9.6 |
| Morgenduft (Dallago) | MOR_1 | 2011 | Maso Part | 27/09/2011 | 30/11/2011 | 305.5 | 12.3 | 4.2 |
| Pilot | PIL_0 | 2010 | Giaroni | 15/09/2010 | 17/11/2010 | 225.3 | 12.5 | 9.7 |
| Pilot | PIL_1 | 2011 | Maso Part | 08/09/2011 | 09/11/2011 | 205.8 | 13.3 | 8.0 |
| Pinova | PNV_0 | 2010 | Maso Maiano | 28/09/2010 | 24/11/2010 | 221.8 | 16.6 | 9.4 |
| Pinova | PNV_1 | 2011 | Maso Part | 13/09/2011 | 16/11/2011 | 231.7 | 12.7 | 5.7 |
| Pinova (Roho) | RHO | 2011 | Maso Maiano | 15/09/2011 | 23/11/2011 | 222.2 | 13.3 | 8.2 |
| Red Chief | RCF_0 | 2010 | Giaroni | 07/09/2010 | 03/11/2010 | 268.7 | 12.7 | 4.2 |
| Red Chief | RCF_1 | 2011 | Maso Part | 31/08/2011 | 26/10/2011 | 299.3 | 11.2 | 4.1 |
| Red Delicious | RED_0 | 2010 | Maso Maiano | 20/09/2010 | 17/11/2010 | 222.3 | 10.0 | 4.1 |
| Red Delicious | RED_1 | 2011 | Maso Part | 31/08/2011 | 26/10/2011 | 277.7 | 11.5 | 3.3 |
| Red Spur (Jeromine) | JER | 2011 | Maso Part | 31/08/2011 | 02/11/2011 | 301.4 | 12.7 | 4.2 |
| Renetta Bianca | RNB_0a | 2010 | Giaroni | 07/09/2010 | 03/11/2010 | 318.8 | 14.6 | 13.8 |
| Renetta Bianca | RNB_0c | 2010 | Maso Maiano | 20/09/2010 | 19/11/2010 | 257.9 | n.d. | 9.4 |
| Renetta Bianca | RNB_1b | 2011 | Maso Part | 31/08/2011 | 26/10/2011 | 296.7 | 12.3 | 11.0 |
| Renetta Bianca | RNB_1c | 2011 | Maso Maiano | 13/09/2011 | 11/11/2011 | 256.9 | 13.8 | 13.4 |
| Renetta Grigia | RNG_b | 2011 | Maso Part | 31/08/2011 | 02/11/2011 | 310.1 | 13.0 | 9.2 |
| Renetta Grigia | RNG_c | 2011 | Maso Maiano | 13/09/2011 | 16/11/2011 | 282.3 | 14.0 | 15.0 |
| Rubens | RUB_0 | 2010 | Maso Maiano | 21/09/2010 | 19/11/2010 | 191.4 | 13.9 | 6.0 |
| Rubens | RUB_1 | 2011 | Maso Part | 08/09/2011 | 09/11/2011 | 243.8 | 11.0 | 4.0 |
| Stayman | STY_0 | 2010 | Maso Part | 04/10/2010 | 07/12/2010 | 289.9 | 12.4 | 7.2 |
| Stayman | STY_1 | 2011 | Maso Part | 22/09/2011 | 30/11/2011 | 309.2 | 12.3 | 6.0 |
| Topaz | TOP_0 | 2010 | Maso Maiano | 28/09/2010 | 24/11/2010 | 236.2 | 14.3 | 13.2 |
| Topaz | TOP_1 | 2011 | Maso Part | 15/09/2011 | 23/11/2011 | 250.1 | 12.4 | 10.7 |

a: average value from 20 fruit, expressed as grams.
b: average value from 12 fruit, expressed as percentage.
c: average value from 12 fruit, expressed as meq. malic acid/100g juice.

**Table 2:** Sensory lexicon used by the sensory panels in 2010 and 2011.

| Category | Descriptor | Definition[a] |
|---|---|---|
| Appearance | Green flesh | Flesh green depth |
| Appearance | Yellow flesh | Flesh yellow depth |
| Texture | Hardness | Resistance of the sample at the first chews with molars |
| Texture | Juiciness | Amount of juice released during chewing (first three chews) |
| Texture | Crunchiness | Sound (pitch/intensity) produced by the sample during 5 molar chews |
| Texture | Flouriness | Degree of flesh breaking in small and dry fragments/granules during chewing |
| Texture | Fibrousness | Degree of flesh breaking during chewing in thick and fibrous fragments/granules |
| Texture | Graininess | Numbers/size of fragments/granules produced during chewing |
| Flavour | Sweet taste | Sweet taste sensation |
| Flavour | Sour taste | Sour taste sensation |
| Flavour | Astringency | Tactile dryness sensation in the mouth (at the end of mastication) |

a: Details about evaluation procedures and reference standards in Corollaro et al. (2013).

**Table 3:** Mechanical and acoustic parameters calculated on the force and sound curves, respectively, coming from TA-XT texture analyser analysis on apple samples.

| Category | Code | Description |
|---|---|---|
| Mechanical | F1 | Yield Force |
| Mechanical | F2 | Max Force |
| Mechanical | F3 | Final Force |
| Mechanical | FP | N° Force Peaks |
| Mechanical | A | Area |
| Mechanical | FLD | Force Linear Distance |
| Mechanical | Y | Young's Module |
| Mechanical | F4 | Mean Force |
| Mechanical | F1-F3 | Delta Force |
| Mechanical | F1/F3 | Force Ratio |
| Mechanical | P/D | Peaks/Distance |
| Mechanical | LD/D | Linear Distance/Distance |
| Acoustic | AUXP | N° Acoustic Peaks |
| Acoustic | AUX1 | Max Acoustic Pressure |
| Acoustic | AUX2 | Mean Acoustic Pressure |
| Acoustic | AUXLD | Acoustic Linear Distance |

**Table 4:** PLS-1 models and validated $R^2$ values, calculated for each sensory attribute, using different series of instrumental data for the development of the models. “Box-Cox Transformation” column refers to the use of the transformed (Y) or untransformed (N) data. “Nr. components” refers to the number of components used for achieving the best prediction model. “Type of instrumental data” refers to the parameters used for developing the model: “Chroma + Chemical” is for L*a*b, SSC and titratable acidity data; “TA-XT” is for mechanical and acoustic texture analyser data; “All” is for the entire instrumental data-set.

| **Attribute** | **Type of instrumental Data** | **Box Cox transformation** | **PLS-1 model** | **$R^2$** | **Nr. Components** |
|---|---|---|---|---|---|
| Green flesh | Color + Chemical | N | y = 0,4339x + 8,6972 | 0,4911 | 1 |
| Yellow flesh | Color + Chemical | N | y = 0,8629x + 5,6267 | 0,9019 | 2 |
| Hardness | TA-XT | N | y = 0,8624x + 5,5972 | 0,8770 | 1 |
| Juiciness | All | N | y = 0.7896x + 9,8693 | 0,8115 | 2 |
| Crunchiness | TA-XT | N | y = 0,8470x + 6,6765 | 0,8532 | 1 |
| Flouriness | TA-XT | N | y = 0,7838x + 6,7745 | 0,7867 | 2 |
| Fibrousness | TA-XT | N | y = 0,7919x + 6,9158 | 0,8003 | 1 |
| Graininess | TA-XT | N | y = 0,7771x + 8,0034 | 0,7696 | 2 |
| Sweet taste | Color + Chemical | Y | y = 0,8352x + 6,9063 | 0,8184 | 3 |
| Sour taste | Color + Chemical | Y | y = 0,8647x + 4,8504 | 0,8876 | 2 |
| Astringency | All | N | y = 0,6109x + 8,3952 | 0,5280 | 5 |

**Fig. 1:** Correlation loading plot (a) and score plot (b) from Principal Component Analysis (PCA) on sensory data-set. For apple samples coding, see “Code” column in Table 1.

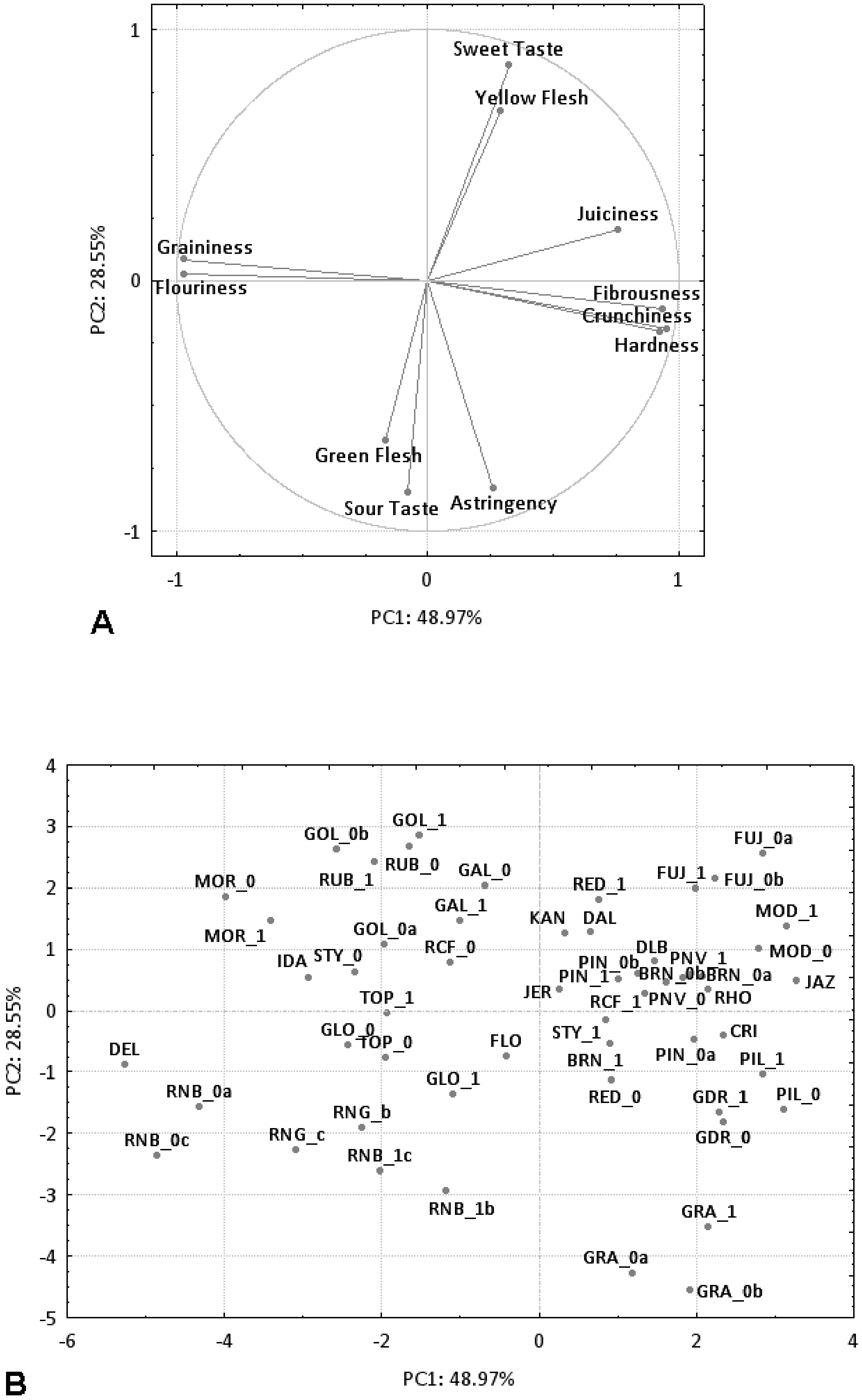


**Fig. 2:** x and y loading plot from PLS-2 analysis on instrumental and sensory data, to predict apple sensory profiles from instrumental parameters (X-var = 62%; Y-var = 57%). Instrumental parameters are reported in regular font, sensory attributes in italic. For texture analyser parameters coding, see “Code” column in Table 3.

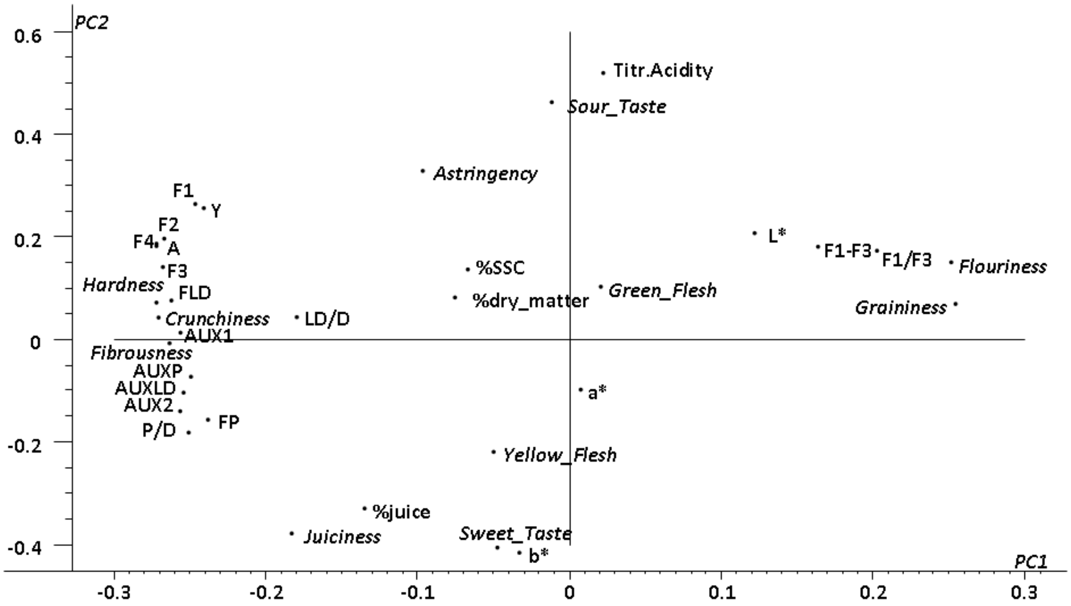
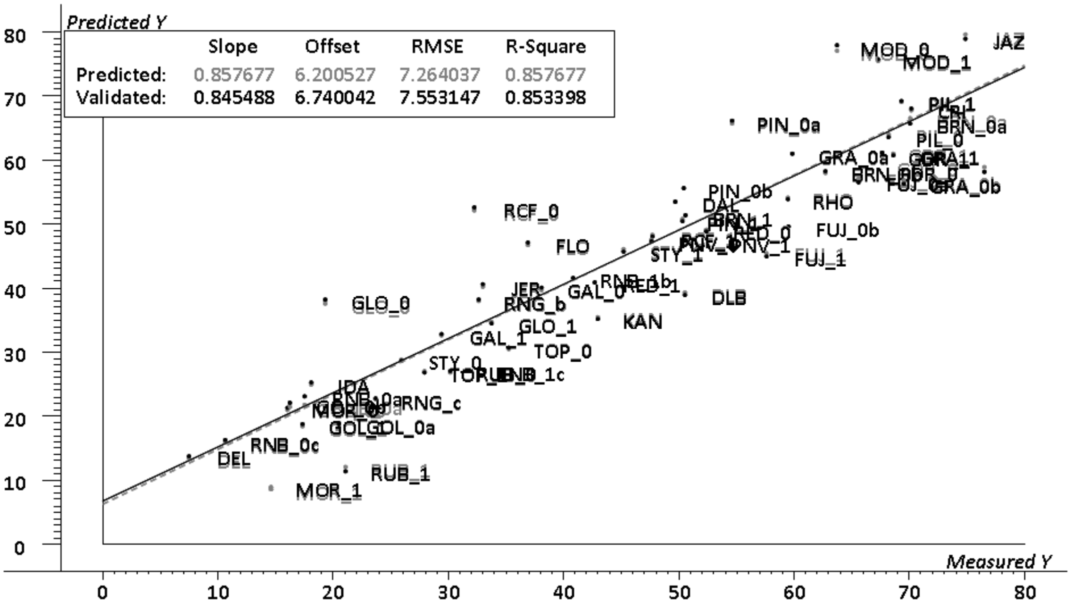


**Fig. 3:** Measured vs. predicted plot from PLS-1 model developed for crunchiness sensory attribute from acoustic and mechanical data from texture analyser analysis. Slope, offset, Root Mean Square Error (RMSE) and $R^2$ (R-Square) are reported for predicted and validated interpolation lines, which are shown as dotted and continuous lines, respectively.



|  | Slope | Offset | RMSE | R-Square |
|---|---|---|---|---|
| Predicted: | 0.857677 | 6.200527 | 7.264037 | 0.857677 |
| Validated: | 0.845488 | 6.740042 | 7.553147 | 0.853398 |

# *Chapter IV*

# SENSORY PROPERTIES OF APPLES GROWN IN DIFFERENT CLIMATIC CONDITIONS

## INTRODUCTION

In the range of pre-harvest factors affecting fruit quality, altitude is one of the most important, determining differences in physiological mechanisms of fruit growth, ripening stage and chemical composition, as demonstrated by several studies (Ferrandino et al., 1999; Comai et al., 2005; Singh et al., 2006; Aslantas and Karakurt, 2007). All the factors above are related to final fruit texture and flavour characteristics. However, to our knowledge, only a few studies applied sensory analysis to evaluate the real perceivable differences between apples grown at different altitudes. Eccher Zerbini et al. (1978) described the differences existing between apples grown at 3 and 350m a.s.l. in terms of texture, acidity, sweetness and aroma by means of a trained panel. Paprštein et al. (2006) studied fruit chosen from orchards in four climatically different locations (about 200, 300, 400 and 500 m a.s.l.) by asking panels of consumers to score their liking for several sensory attributes related to appearance, flavour and texture. None of them provided details about the sensory evaluation procedures, it is even possible that no rigorous protocols for sensory analysis were applied in any case. Indeed, in the context of the studies on pre-harvest factors, for long time sensory analysis has not been considered a useful and convenient way to obtain information on fruit quality. Of course, sensory analysis is expensive, needs time and resources. Nevertheless, its fundamental role in the evaluation of food quality and consumer perception of food properties has been recognized. Many authors underlined how analytical measurements are not always adequate to substitute for sensory evaluation in screening of food products and that human assessment should be the standard against which instrument readings should be calibrated (Hampson et al., 2000; Bourne, 2002; Harker et al. 2002a).

Thus, in this work the application of a detailed protocol for quantitative descriptive analysis of apples (Malus × domestica Borkh.) is proposed to study the perceivable differences between apples grown in different climatic conditions. Instrumental analyses were also applied to confirm the results provided by the sensory description and to give interpretation to the sensory differences.

## MATERIALS AND METHODS

### *Plant material*

Goden Delicious apples were provided by Laimburg Research Centre for Agriculture and Forestry (Vadena, Bolzano, Italy), coming from three different orchards at 600m a.s.l. (low altitude, La, Lb, Lc) and three around 1000m a.s.l. (high altitude, Hd, He, Hf), all applying the same growing practices. From each orchard, in 2011 apple season three different harvest times were considered: T0, chosen by measuring basic parameters (firmness, % soluble solid concentration, titratable acidity, starch index); T1, one week later; T2, three weeks after T0. Informations about the agronomical features are reported in Table 1. The fruit were stored for five months in refrigerated ultra low oxygen atmosphere condition. Then, they were kept for 24h at room temperature before the analyses.

### *Sensory analysis*

Sensory analysis was performed based on the quantitative descriptive analysis method (Stone and Sidel, 2004a) in a sensory laboratory equipped with 22 individual booths under artificial red light by a trained panel composed of 17 people (6 males and 11 females), all employees at Fondazione Edmund Mach (San Michele all’Adige, Trento, Italy). The sensory vocabulary included six attributes for texture, taste, overall odour, overall retro-nasal flavour and astringency. Moreover, 6 specific attributes for odour and retro-nasal flavour were scored (Table 2).

Samples were cut in small flesh cylinders, treated with an antioxidant solution (0.2% ascorbic acid; 0.2% citric acid; 0.5% calcium chloride), and presented in randomised balanced order, labelled with three digit codes. Details about the procedures are reported in Corollaro et al. (2013).

### *Texture analysis*

Analyses were performed on flesh cylinders coming from the same fruit provided to the sensory panel.

Texture measurements were performed by a TA-XT texture Analyzer equipped with an acoustic envelop detector device (Stable MicroSystem Ltd., Godalming, UK), using a 4 mm probe to compress the samples. The measurements were taken on eight cylinders from eight different fruit, following the method by Costa et al. (2011): twelve mechanical and four acoustic parameters were calculated (Table 3). The measure of percentage of extractable juice (% juice) was mechanically performed by squeezing of eight flesh cylinders from eight different fruit and calculated as weight difference.

### *Basic chemical composition*

Soluble solid concentration (SSC) and titratable acidity were measured in duplicate on the juice squeezed from eight cylinders from different fruit, by a DBR35 refractometer (XS Instruments, Poncarale, Brescia, Italy) and a Compact Titrator (Crison Instruments S.A., Alella, Barcelona, Spain), respectively. NaOH 0.1N was used to titrate the samples to pH 8.16. The results were calculated as mail acid milliequivalents in 100g juice.

### *Cell anatomy analysis*

The analysis of anatomical features was performed on fruit from T0 through the method described by Goffinet et al. (1995) for apple cell counting. Each fruit was cut along the equator line. Two wedge-shaped sectors were re-cut by a razor blade along the longer and the shorter radius of the cortex. Three photographs at 10x magnification were taken at one fourth, half and three fourth of each radius by a Leica DMLB light microscopy equipped with a DC 300F camera supported by IM1000 Image Manager software (Leica Microsystems AG, Heerbrugg, Switzerland). The photos were analysed by ImageJ 1.45s software (USA), by applying a grid of 11000 pixel2 per square and counting cells and intercellular spaces inside a grid composed of nine rows and eleven columns. The sample preparation procedure for such measurement was incompatible with sensory evaluation procedure, thus, different fruit were used for anatomical measures.

### *Statistical analysis*

Two-factor ANOVA on sensory and instrumental data, considering altitude and time of harvest as experimental factors and one-way ANOVA on cell counting measurements data were performed by the STATISTICA 9.1 software (StatSoft, Inc., USA). *P*-values equal or lower than 0.05 were considered significant. Generalized Procrustes Analysis (GPA) was performed on sensory data to study the sensory space by Senstools 3.1.6 software (OP&P Product Research BV, Utrecht, the Netherlands).

## RESULTS AND DISCUSSION

### *Sensory profiling*

The two-factor ANOVA on sensory data showed significant differences between the two altitudes and among the three times of harvest for many sensory attributes (Table

4). As for time of harvest, samples from T2 showed lower intensity for hardness, crunchiness, fibrousness, and sour taste, and higher intensity for flouriness and graininess. As for altitude factor, samples from low altitude were perceived as juicier, crunchier and more fibrous than samples from high altitude, which were more floury and grainy. The sensory differences are well described by the bi-plots from the GPA performed on the general and the odour/flavour sensory data-sets (Figs. 1a and 1b). In Figure 1a, the samples are distributed along the first component as they pass from T0 to T2, from high hardness and crunchiness values to high flouriness and graininess. The distribution along the second component is related to the sweet and sour tastes, with samples having high sweetness in the lower part of the plot. In general, samples from high altitude appeared to be located in the upper side of the plot, excepted for Hf samples. On the contrary, samples from low altitude appeared to be located in the lower part. Thus, samples from low altitude were generally described as sweeter and juicier than samples from high altitude, appearing more sour and astringent. We have no clear explanation for Hf samples behaviour. It is possible that the relatively low crop load of Hf orchard made the fruit reach a ripening stage and textural/chemical properties closer to the fruit from low altitude orchards (see Table 1 for crop load details). Actually, it is demonstrated that crop load is negatively associated to fruit size (Henriod et al., 2011; Saei et al., 2011). Moreover, apple fruit size has been associated to maturity levels at harvest (Koorey and Brookfield, 1999).

The odour and flavour profile shown in Figure 1b did not show a sample distribution related to the altitude, only to time of harvest. The samples from T2 are all located in the left part of the plot, showing high intensities for pear, banana and vanilla odour and flavour, while samples from T0 and T1 were described as having mainly grass and lemon odour/flavour. Significant differences were perceived for pear and banana odours and for banana flavour, which were found to have higher intensity in T2 than T0. Lemon flavour was higher in T0 than T2. In their work on ‘Golden Delicious’ apples from three different altitudes (350, 750, 1000 m a.s.l.), Ferrandino et al. (1999) found that a different volatile compounds (VOCs) profile was developed by the fruit in the different climatic environments. They found a higher development of volatiles in fruit from 1000 m a.s.l. Actually, VOCs emission of our fruit was analysed by gas-chromatography and mass spectrometry, but these data are not included in this paper. However, in our study, from a sensory point of view, altitude seemed not to affect volatiles perception. Only honey odour showed significant differences, with samples from high altitude having higher intensity than those from low altitude.

### *Texture profiling and basic composition*

The two-factor ANOVA on instrumental data confirmed the sensory description. Time of harvest showed differences for titratable acidity and texture analyser parameters, both mechanical and acoustic. In particular, samples from T2 were found to have lower acid concentration than T0 and T1. Mechanical and acoustic parameters from texture analyser measures showed a gradual decrease as time of harvest passed from T0 to T2. Significant altitude effect was seen for texture parameters and for SSC, with samples from low altitude having higher mechanical and acoustic response, higher percentage of extractable juice and higher SSC (Fig. 2). Three mechanical parameters also showed interaction between the two factors, suggesting that at different altitudes the structural properties of fruit tissue can have a different evolution during fruit ripening (Table 5).

However, it is important to consider that in our study only one year of fruit production was considered. Thus, preliminary results are discussed here and it is not possible to extrapolate general considerations.

### *Cell anatomy characterisation*

One-way ANOVA on anatomical data showed that fruit from low altitude had a higher amount of cells and higher percentage of intercellular spaces (Figs. 3a and 3b). Even if fruit weight was not significantly different, the volume of fruit from low altitude was higher than fruit from high altitude (Fig. 3c). Thus, it is possible to suppose that cell division was longer in fruit from low altitude, with higher number of cell replications as compared to fruit from high altitude. This could have been caused an increase in fruit expansion at low altitude. Warrington et al. (1999) demonstrated that different range of temperatures during fruit growth (from 10 to 40 days after full bloom) caused differences in fruit volume, weight and quality traits in several apple varieties. Fruit were found to be bigger when temperatures were higher. They also showed higher SSC, even if a decrease in flesh firmness was found as the temperatures increased. Stanley et al. (2000) suggested that early season temperatures are important in determining final fruit weight, while late season temperatures are more likely to influence ripening physiology than fruit growth. This is explained by the fruit growth mechanism, that shows an early exponential cell division phase, lasting for the first week; than a phase of contemporary cell division and cell expansion follows, and lasts for about 3-4 weeks; and finally a phase of cell expansion only characterises the rest of the season (Lakso and Goffinet, 2013). We can suppose that the average temperatures at 1000 m a.s.l. were lower than those at 600 m a.s.l., since, in our study no data about heat accumulation during fruit growth in the six orchards are available.

The anatomical data were consistent with the sensory and instrumental description. The higher the number of cells, the higher the force required to compress the sample, which is confirmed by texture analyser measurements. Moreover, higher number of cells means higher amount of cell walls crushing during compression. The cell wall rupture and the expansion of the liquid content under pressure are responsible for the sound emitted by wet foods when they crush (Duizer, 2001). Moreover, fruit from low altitude showed higher percentage of air spaces, and the amount of air spaces is related to the acoustic response: the higher the amount of air spaces, the higher the sound, because the expansion of the cell liquid in the surrounding empty spaces causes noise emission. The sound produced when food crushes is strongly related to the sensory perception of crunchiness (Fillion and Kilcast, 2002). Our sensory data were in agreement with such observation, since crunchiness intensity was higher in samples from low altitude.

## CONCLUSIONS

Even if the samples from the four orchards were all harvested in dependence of same basic parameters (measured at T0), important differences were found not only as a consequence of time of harvest, but also related to the altitude: apples from high altitude show a lower fruit volume, with a lower amount of cells and intercellular spaces, probably due to different early season temperatures causing different cell division patterns. That was responsible for different texture properties, and such differences were perceivable by human senses. Although this study was performed on fruit from only one year, these preliminary results suggest that differences in terms of anatomical and structural features developed by apples grown in different climatic conditions can be perceived by human senses and that the sensory-instrumental tool here applied provided useful information to describe such differences. Thus, a proper sensory evaluation of apple fruit from very different locations should be always considered and applied in order to have a reliable description of what consumers will perceive in the final product.

**Table 1:** Agronomical data from the six orchards under study. T0, T1 and T2 refer to the harvest dates here considered. In this study, samples from orchards around 600m a.s.l. are considered from low altitude; samples from orchards around 1000m a.s.l. are considered from high altitude.

| Code | m a.s.l. | Year planting | Light exposure | % slope | Crop load (t/ha) | T0 | T1 | T2 |
|---|---|---|---|---|---|---|---|---|
| La | 652 | 2010 | N | 8.5 | 85.3 | 19/09/2012 | 25/09/2012 | 10/10/2012 |
| Lb | 656 | 2009 | N | 11.0 | 95.4 | 19/09/2012 | 25/09/2012 | 10/10/2012 |
| Lc | 580 | 2003 | N | 11.4 | 98.2 | 19/09/2012 | 25/09/2012 | 10/10/2012 |
| Hd | 1070 | 2002 | S | 9.3 | 91.4 | 26/09/2012 | 02/10/2012 | 16/10/2012 |
| He | 1040 | 2010 | S | 13.2 | 52.4 | 26/09/2012 | 02/10/2012 | 16/10/2012 |
| Hf | 1070 | 2010 | S | 15.8 | 69.6 | 26/09/2012 | 02/10/2012 | 16/10/2012 |

**Table 2:** Sensory vocabulary used by the trained panel.

| Category | Descriptor | Definition |
|---|---|---|
| Texture | Hardness | Resistance of the sample at the first chew with molars |
| Texture | Juiciness | Amount of juice released during chewing (first three chews) |
| Texture | Crunchiness | Sound (pitch/intensity) produced by the sample during 5 molar chews |
| Texture | Flouriness | Degree of flesh breaking in small and dry fragments/granules during chewing |
| Texture | Fibrousness | Degree of flesh breaking during chewing in thick and fibrous fragments/granules |
| Texture | Graininess | Numbers/size of fragments/granules produced during chewing |
| Flavour | Sweet taste | Sweet taste sensation |
| Flavour | Sour taste | Sour taste sensation |
| Flavour | Astringency | Tactile dryness sensation in the mouth (at the end of mastication) |
| Flavour | Overall Odour | Overall odour sensation |
| Flavour | Overall Flavour | Overall flavour sensation |
| Flavour | Pear | Specific odour (Od) or retro-nasal flavour (Fl) sensation |
| Flavour | Banana | Specific odour (Od) or retro-nasal flavour (Fl) sensation |
| Flavour | Lemon | Specific odour (Od) or retro-nasal flavour (Fl) sensation |
| Flavour | Grass | Specific odour (Od) or retro-nasal flavour (Fl) sensation |
| Flavour | Vanilla | Specific odour (Od) or retro-nasal flavour (Fl) sensation |
| Flavour | Honey | Specific odour (Od) or retro-nasal flavour (Fl) sensation |

**Table 3:** Mechanical and acoustic parameters from the curves developed by texture analyser compression measurements, following the method by Costa et al. (2011).

| Category | Code | Description |
|---|---|---|
| Mechanical | F1 | Yield Force |
| Mechanical | F2 | Max Force |
| Mechanical | F3 | Final Force |
| Mechanical | FP | N° Force Peaks |
| Mechanical | A | Area |
| Mechanical | FLD | Force Linear Distance |
| Mechanical | Y | Young's Module |
| Mechanical | F4 | Mean Force |
| Mechanical | F1-F3 | Delta Force |
| Mechanical | F1/F3 | Force Ratio |
| Mechanical | P/D | Peaks/Distance |
| Mechanical | LD/D | Linear Distance/Distance |
| Acoustic | AUXP | N° Acoustic Peaks |
| Acoustic | AUX1 | Max Acoustic Pressure |
| Acoustic | AUX2 | Mean Acoustic Pressure |
| Acoustic | AUXLD | Acoustic Linear Distance |

**Table 4:** Mean values and *p*-values from two-factor ANOVA on sensory data, considering harvest time and altitude as factors. *P*-values lower than 0.05 are considered significant. Mean values followed by same letters are not significantly different.

| | **Time of harvest** | | | | **Altitude** | | | **Time of harvest*Altitude** |
|---|---|---|---|---|---|---|---|---|
| **Attribute** | **T0** | **T1** | **T2** | ***p*-value** | **Low** | **High** | ***p*-value** | ***p*-value** |
| Overall Odour | 43.0 | 47.9 | 50.0 | 0.088 | 46.0 | 48.0 | 0.437 | 0.863 |
| Od-Pear | 17.8 a | 18.4 ab | 24.9 b | 0.035 | 18.1 | 22.6 | 0.067 | 0.148 |
| Od-Banana | 12.4 a | 15.1 ab | 20.5 b | 0.026 | 13.8 | 18.3 | 0.071 | 0.614 |
| Od-Lemon | 7.5 | 7.1 | 5.7 | 0.581 | 7.0 | 6.5 | 0.765 | 0.457 |
| Od-Grass | 6.1 | 6.9 | 5.8 | 0.779 | 6.7 | 5.8 | 0.516 | 0.766 |
| Od-Vanilla | 5.4 | 6.6 | 6.5 | 0.739 | 5.6 | 6.7 | 0.424 | 0.108 |
| Od-Honey | 5.1 | 5.6 | 5.7 | 0.923 | 3.9 a | 7.0 b | 0.020 | 0.657 |
| Hardness | 56.6 b | 48.9 b | 25.0 a | 0.000 | 46.0 | 41.0 | 0.069 | 0.466 |
| Juiciness | 44.0 | 41.3 | 37.0 | 0.088 | 43.8 b | 37.7 a | 0.020 | 0.606 |
| Crunchiness | 57.2 b | 50.7 b | 25.6 a | 0.000 | 49.5 b | 39.5 a | 0.001 | 0.378 |
| Flouriness | 4.9 a | 10.0 a | 30.8 b | 0.000 | 11.9 a | 18.5 b | 0.001 | 0.249 |
| Fibrousness | 46.0 b | 38.7 b | 16.0 a | 0.000 | 38.2 b | 28.9 a | 0.004 | 0.936 |
| Graininess | 12.9 a | 18.0 a | 35.3 b | 0.000 | 19.1 a | 25.0 b | 0.014 | 0.467 |
| Sweet Taste | 38.3 | 37.7 | 44.1 | 0.091 | 40.4 | 39.7 | 0.783 | 0.771 |
| Sour Taste | 40.8 b | 40.1 b | 24.3 a | 0.000 | 33.4 | 36.7 | 0.242 | 0.517 |
| Astringency | 27.0 b | 25.8 ab | 17.6 a | 0.040 | 22.2 | 24.7 | 0.449 | 0.980 |
| Overall Flavour | 45.9 | 46.1 | 47.2 | 0.905 | 45.4 | 47.4 | 0.430 | 0.572 |
| Fl-Pear | 12.1 | 14.5 | 15.3 | 0.368 | 14.1 | 13.8 | 0.886 | 0.308 |
| Fl-Banana | 6. a | 9.0 ab | 11.7 b | 0.026 | 7.6 | 10.2 | 0.130 | 0.847 |
| Fl-Lemon | 16.1 b | 15.8 b | 9.4 a | 0.018 | 12.5 | 15.0 | 0.247 | 0.751 |
| Fl-Grass | 10.1 | 10.7 | 6.5 | 0.154 | 7.9 | 10.3 | 0.221 | 0.408 |
| Fl-Vanilla | 4.3 | 5.0 | 4.8 | 0.872 | 4.4 | 4.9 | 0.640 | 0.729 |
| Fl-Honey | 6.1 | 5.7 | 5.9 | 0.976 | 6.1 | 5.7 | 0.757 | 0.762 |

**Table 5:** Mean values and *p*-values from two-factor ANOVA on instrumental data, considering harvest time and altitude as factors. *P*-values lower than 0.05 are considered significant. Mean values followed by same letters are not significantly different. For texture analyser parameters coding, see Table 3.

| | **Time of harvest** | | | | **Altitude** | | | **Time of harvest*Altitude** |
|---|---|---|---|---|---|---|---|---|
| **Parameter** | **T0** | **T1** | **T2** | ***p*-value** | **Low** | **High** | ***p*-value** | ***p*-value** |
| SSC | 15.4 | 14.5 | 15.0 | 0.167 | 16.2 b | 13.7 a | 0.000 | 0.092 |
| Titratable acidity | 8.0 a | 7.3 a | 5.9 b | 0.000 | 7.3 | 6.9 | 0.323 | 0.637 |
| F1 | 10.8 c | 8.9 b | 7.5 a | 0.000 | 9.4 | 8.8 | 0.057 | 0.076 |
| F2 | 12.2 c | 10.0 b | 8.5 a | 0.000 | 10.6 b | 9.9 a | 0.012 | 0.037 |
| F3 | 9.2 c | 7.4 b | 6.4 a | 0.000 | 7.9 b | 7.4 a | 0.033 | 0.050 |
| FP | 25.4 b | 24.7 ab | 23.5 a | 0.007 | 25.1 b | 24.0 a | 0.026 | 0.506 |
| A | 836.2 c | 700.4 b | 592.5 a | 0.000 | 733.8 b | 687.4 a | 0.005 | 0.022 |
| FLD | 104.7 c | 102.0 b | 99.4 a | 0.000 | 102.8 b | 101.3 a | 0.002 | 0.862 |
| Y | 1.5 b | 1.4 b | 1.2 a | 0.000 | 1.4 | 1.3 | 0.413 | 0.350 |
| F4 | 9.7 c | 8.1 b | 6.9 a | 0.000 | 8.5 b | 8.0 a | 0.005 | 0.022 |
| F1-F3 | 1.7 | 1.5 | 1.1 | 0.154 | 1.5 | 1.4 | 0.709 | 0.827 |
| F1/F3 | 1.2 | 1.2 | 1.2 | 0.888 | 1.2 | 1.2 | 0.892 | 0.905 |
| P/D | 2.1 b | 2.0 b | 1.9 a | 0.000 | 2.0 b | 1.9 a | 0.009 | 0.519 |
| LD/D | 8.6 c | 8.4 b | 8.0 a | 0.000 | 8.4 b | 8.2 a | 0.007 | 0.854 |
| AUXP | 39.7 c | 25.3 b | 16.1 a | 0.000 | 36.8 b | 17.5 a | 0.000 | 0.208 |
| AUX1 | 64.9 c | 61.7 b | 59.6 a | 0.000 | 63.0 b | 61.1 a | 0.000 | 0.637 |
| AUX2 | 47.6 c | 47.0 b | 46.0 a | 0.000 | 47.2 b | 46.5 a | 0.002 | 0.742 |
| AUXLD | 5461.7 c | 4624.9 b | 3854.1 a | 0.000 | 5281.6 b | 4024.3 a | 0.000 | 0.803 |
| % juice | 37.2 | 37.7 | 41.0 | 0.247 | 40.8 b | 36.5 a | 0.037 | 0.970 |

**Fig. 1:** Bi-plot showing the first two components from GPA performed on overall (a; Dim.1: 49%; Dim.2: 10%) and odour/flavour (b; Dim.1: 21%; Dim.2: 15%) sensory data-sets. Samples from low altitude are indicated by full markers; samples from high altitude by empty markers. Circle markers are for T0; triangles are for T1; squares are for T2.

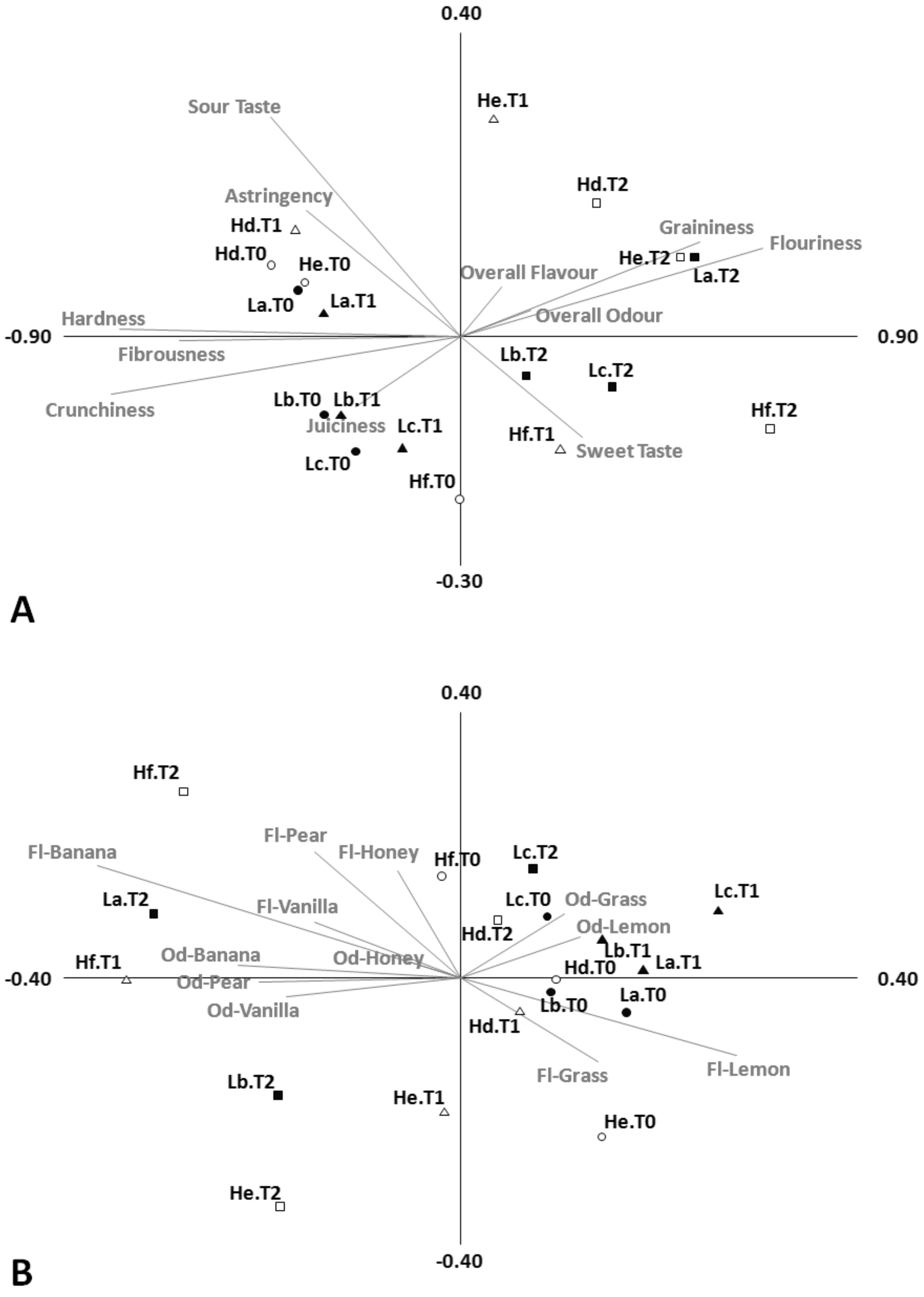


**Fig. 2:** Mean values for maximum force (a) and maximum acoustic response (b) from texture analyser measurements and percentage of mechanically extractable juice (c) on samples from high and low altitude. Different letters on the bars refer to significant differences.

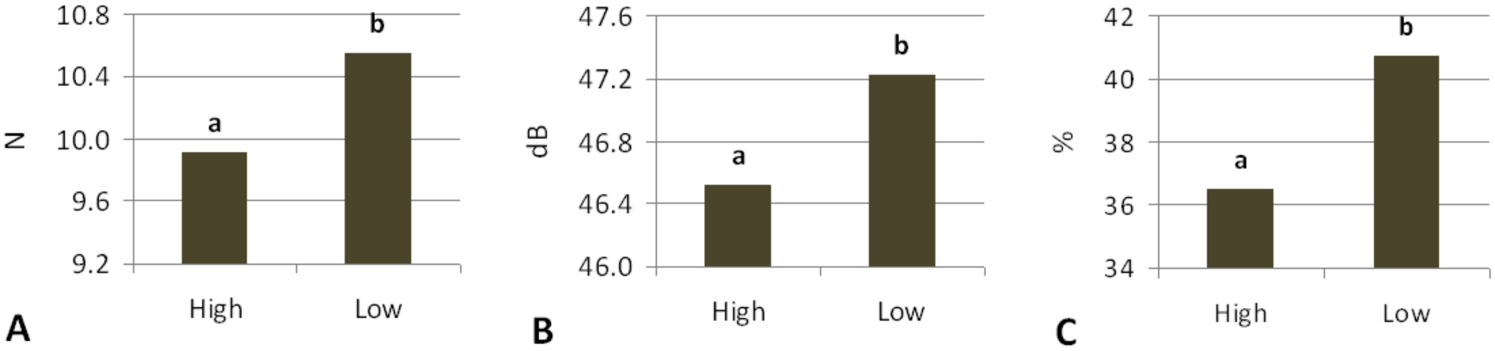
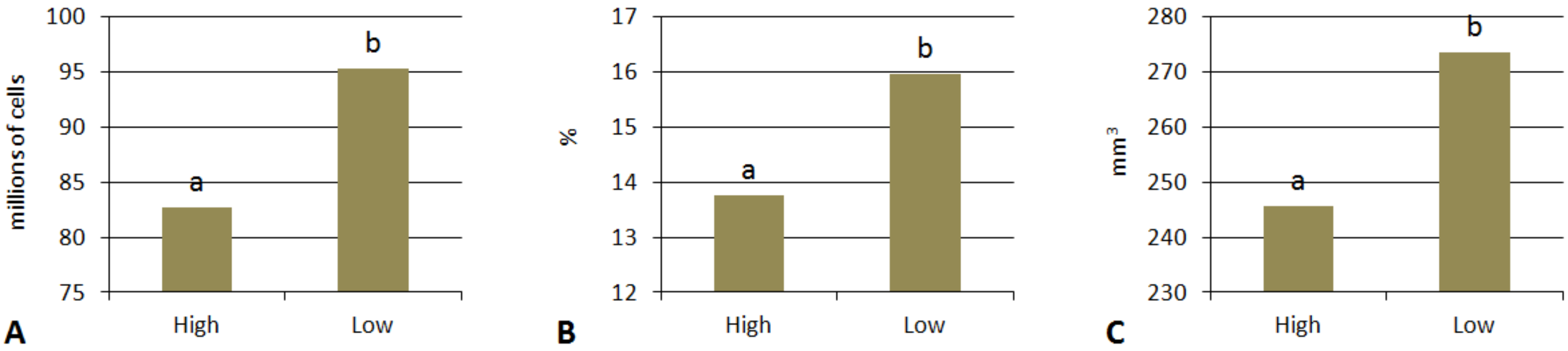


**Fig. 3:** Mean values for number of cells/fruit (a), percentage of air spaces (b) and fruit volume (c) from anatomical measures on fruit from high and low altitude. Different letters on the bars refer to significant differences.



# *Chapter V*

# THINNING VIA SHADING AND THE USE OF PHOTOSELECTIVE NETS: THE INFLUENCE ON SENSORY QUALITY IN APPLES

## INTRODUCTION

In apple production, meeting market demand while providing fruit of the highest quality is a difficult challenge. To achieve optimum fruit production, high fruit numbers are needed at fruit set, which are then thinned to the desired level. Thinning is therefore key to improve yield and quality in apple (Byers, 1990; Link, 2000). Crop management practices and pre-harvest treatments influence product quality both at harvest and during storage (Sams, 1999; Johnston et al., 2002). Crop load has been shown to affect fruit firmness and sensory properties. Most studies show better quality fruit from low compared to higher crop load trees (Delong et al., 2006; Baugher and Schupp, 2010; Henriod et al., 2011). To reduce crop load, growers may hand-remove fruit but, due to cost and time, the application of phytochemicals which cause fruit drop is widely used, normally followed by hand-thinning adjustment to optimise fruit load. Avoiding use of chemicals is a general goal in fruit growing, therefore an alternative method has been proposed, based on heavy shading of trees, to virtually stop net carbon assimilation which leads to abscission (Zibordi et al., 2009; Morandi et al., 2011). Although it still presents technical difficulties, this approach has been shown to be economically viable for those organic growers who hand-thin their fruit (Widmer et al., 2008). Photoselective coloured nets have been developed to promote specific physiological responses by differential spectral transmission of solar radiation (Shahak et al., 2004). Bastías et al. (2012) found slight differences in apple fruit coming from trees grown under different coloured nets, with larger fruit size under blue, compared to white, grey, or red nets. They attributed this response to an increase in leaf photosynthesis induced by the higher blue/red wavelength ratio available under the blue net, that may have benefitted fruit growth.

Conflicting reports have been published about the quality of apples coming from shading treatments or orchards under hail nets: Solomakhin and Blanke (2010) and Amarante et al. (2011) found poorer quality in apples coming from hail net-covered orchards, with lower firmness, lower soluble solids and acid content, lower vitamin C content, and a consequent reduction of fruit shelf life, compared to fruit from unprotected orchards. For this reason, some authors suggest the use of reflective foil or mulch covering the grass alleyways to contrast adverse effects on fruit quality due to the light availability reduction caused by hail nets (Jakopic et al., 2007; Solomakhin and Blanke, 2007). On the other hand, Widmer et al. (2008) found good results in terms of basic fruit quality parameters (fruit weight, firmness, soluble solid content), which were comparable to chemically- or hand-thinned trees.

Until now, sensory science has never been applied to evaluate the eating quality of fruit produced under altered light microclimates. Any analysis solely based on chemical or physical properties would not suffice for exhaustive fruit quality description, as several sensory attributes may variably interact, influencing and modifying what is really perceived (Harker et al., 2006; Echeverría et al., 2008). Sensory analysis can give meaning to sensory perception, coupling a scientific approach to a language which is close to consumer perception. Descriptive sensory analysis is the best approach to provide a comprehensive and objective description of a product, both qualitative and quantitative (Murray et al., 2001).

This work reports on the quality, as appraised by sensory analysis, of apples coming from two studies of orchard light microclimate manipulation. In the first one, the impact of thinning via shading on sensory quality of apples was assessed by quantitative descriptive analysis coupled to an instrumental characterisation of texture parameters. In the second study, we evaluated the effect of variations in the spectral light composition on the sensory quality of apples grown on trees subjected to different photoselective hail nets. Texture properties and cell anatomical features of fruit samples were studied by instrumental measurements, to give interpretation to any possible sensory differences caused by physiological mechanisms of cell division as affected by light microclimate.

## MATERIALS AND METHODS

### *Fruit material and sample preparation*

Apple fruit were all harvested in 2011 (Table 1). *Experiment 1:* apples were sourced from a mature (2008 planting) ‘Rosy Glow’/M9 commercial orchard near Ravenna, Italy, of approximately one hectare, trained as central leader at a density of 2500 tree/ha (4.0 x 1.0 m). The drip-irrigated orchard is subjected to standard management practices. When fruitlets reached 12 mm diameter (approximately two weeks after full bloom), the entire orchard, minus three rows, was chemically thinned according to standard commercial practice. The central row of the three that were not sprayed was covered with a 90% neutral shading cloth (Bartex 90%; Artes Politecnica Srl, Schio, Vicenza, Italy) applied for one week 30 days after full bloom. Trees were shaded for their entire height. After shade removal the trees received the same management practices as the remainder of the orchard until harvest. The two treatments caused similar fruit drop, as assessed by counting the total number of fruit per tree on ten trees per treatment (data not shown). *Experiment 2:* 'Fuji' apples from a commercial orchard located near Ferrara, Italy, were used. The orchard, trained as slender spindle on M9 rootstock, was planted in 2007 at a spacing of 4.0 x 0.8 m (3125 tree/ha), and is under standard management practices. The 1-ha orchard was divided in sections and covered with photoselective hail nets (ChromatiNet®, Polysack Industries, Negev, Israel) coloured white, red, yellow, and blue; a standard neutral black net was used as control. All these nets reduced light by about 20%; care was applied in their placement to ensure that the test trees were subjected only to the light microclimate caused by a single photoselective net, irrespective of the height of the sun in the sky (Fig. 1). The nets were deployed in the first half of April, immediately after anthesis, till harvest.

At harvest, representative samples, based on the background colour of the fruit were collected from a strip pick of 'Rosy Glow' apple and from the largest pick of each photoselective hail net in 'Fuji', and were stored for three months in normal atmosphere at 2°C, 95% RH. The samples were then prepared as reported in Corollaro et al. (2013): they were kept at room temperature for 24 hours before the analysis, then the flesh from 45 fruit was cut in small cylinders (1.2 cm high; 1.8 cm diameter), treated with an antioxidant solution (0.2% citric acid, 0.2% ascorbic acid, 0.5% calcium chloride) and provided for the sensory analysis in anonymous clear plastic cups (eight cylinders per cup from different fruit), coded with three-digit numbers.

### *Trained panel and sensory analysis*

A trained panel of 10 judges, all volunteers from the Fondazione Edmund Mach (San Michele all'Adige, Trento, Italy), evaluated the apple samples according to a quantitative descriptive method based on a consensus vocabulary with 13 attributes for appearance, texture and flavour. The training and sensory vocabulary are described in Corollaro et al. (2013). Each attribute intensity was rated using a linear scale anchored to 0 (minimum intensity) and 100 (maximum intensity), with a halfway anchor (50). Three replicates per sample were presented in randomised balanced order. Data were acquired by the software FIZZ 2.46A (Biosystemes, Couternon, France). Because of the different harvest period for the two varieties, different sensory analysis sessions were dedicated to Fuji and Rosy Glow samples: For the five Fuji treatments, three different sessions took place (five samples/session), while for the two Rosy Glow treatments, the three replicates were analysed in one session (six samples in total per session).

Panel efficacy was confirmed by analyses on the data-set of 30 apple varieties previously evaluated by the same judges during the period September-December 2011: Judges showed good consistency and discriminant ability for all the texture and taste descriptors (mean *p*-value for all the judges and attributes: 0.019). Overall odour, overall flavour, and astringency gave some problems related to the discriminant ability of one or more judges. Because of such observations, overall odour, overall flavour, and astringency attributes were excluded from the data-set for the following analyses.

### *Instrumental analysis on fruit from sensory analysis*

Instrumental analyses were performed on the same fruit material provided to the sensory panel, with the exception of cell anatomy, which was studied on different fruit, because of an incompatible protocol for sample preparation.

L*a*b components from CIELAB colour space model (Schanda, 2007) were measured on the flesh from each fruit by a Chroma Meter CR-400 colorimeter, supported by the CM-S100w SpectraMagic™ colour data software (Konica Minolta Sensing, Inc., Japan). A sub-sample of flesh cylinders coming from the material provided to the panel was subjected to the other instrumental analyses. The juice squeezed from eight cylinders/sample was used to measure soluble solid concentration (SSC) (DBR35 refractometer, XS Instruments, Poncarale, Brescia, Italy) and titratable acidity (Compact Titrator, Crison Instruments S.A., Alella, Barcelona, Spain) in duplicate. NaOH 0.1 N was used to titrate the juice to pH 8.16. Dry matter concentration was measured by drying of eight flesh cylinders per variety at 105°C until stable weight. A TA-XT texture analyser equipped with an Acoustic Envelope Detector (Stable MicroSystem Ltd., Godalming, UK) was used to analyse the texture properties by compressing with a 4 mm probe ten cylinders/sample (each cylinder coming from a different fruit and corresponding to a replicate). From the mechanical and acoustic profiles/curves, eleven and four parameters were extracted, respectively, following the method described by Costa *et al.* (2011).

### *Instrumental analysis on other fruit*

On 25 fruit/sample cell volume, cell number per fruit, and % fruit intercellular air spaces were assessed following Goffinet et al. (1995). Each fruit was cut along the equator line. Two wedge-shaped sectors were re-cut by a razor blade along the longer and the shorter radius of the cortex. Three photographs at 10x magnification were taken at one fourth, half and three fourth of each radius by a Leica DMLB light microscopy equipped with a DC 300F camera supported by IM1000 Image Manager software (Leica Microsystems AG, Heerbrugg, Switzerland). The photos were analysed by ImageJ 1.45s software (USA), by applying a grid of 11000 pixel$^2$ per square and counting cells and intercellular spaces inside a grid composed of nine rows and eleven columns. From the cell anatomy data, cell packing was computed, defined as the number of cells per unit volume of the fruit cortex parenchyma.

### *Statistical analysis*

The exploratory analysis of sensory data was performed by Generalized Procrustes Analysis (GPA) using the Senstools 3.1.6 software (OP&P Product Research BV, Utrecht, the Netherlands). Sample differences were studied by a three-way mixed ANOVA (for sensory data, considering judge as random factor, and product and replicate as fixed factors) and a one-way ANOVA (for instrumental data) with the

STATISTICA 9.1 software (StatSoft, Inc., USA). *P*-values lower than 0.05 were considered significant. Honestly significant difference (HSD) post-hoc test was performed to study significant differences.

## RESULTS

### *Sensory analysis*

The analysis on the whole data-set, considering both ‘Fuji’ and ‘Rosy Glow’ samples, shows that the panel was able to discriminate between the different apple cultivars. The GPA shows that the first dimension discriminates for apple variety; while the second dimension is able to highlight differences between treatments, in particular for ‘Fuji’ apples (Fig. 2).

Mixed-factorial ANOVA was performed on ‘Rosy Glow’ and ‘Fuji’ data-sets separately, to study differences between the products in the two experiments. In both data-sets, judge effect was significant for all the attributes ($p < 0.001$), except for graininess in experiment 1. Replicate effect was significant for three attributes in both experiments ($p < 0.05$), probably because of the small variability in fruit material (Bavay et al., 2013). Overall, the panel, despite the similarity of the samples, proved to be repeatable and consonant.

*Experiment 1:* Product factor was significant only for the green flesh attribute, with fruit from chemically thinned fruit being more green than ‘Rosy Glow’ from shade-thinning (Table 2).

*Experiment 2:* ‘Fuji’ apples grown under different photoselective hail nets were different for green and yellow flesh, hardness, and sweet taste sensory attributes (Table 2). Apples from the red were less green than fruit from the yellow net. On the contrary for yellow flesh attribute, red net apples were yellower than white and yellow net apples. Fruit from white, red, and blue nets were harder than yellow net fruit. Red net fruit were evaluated as sweeter than blue and yellow net fruit. The other treatments showed intermediate results (Table 2).

### ***Instrumental analysis***

*Experiment 1:* Chemically thinned fruit had a lower acid content ($p < 0.001$). Colorimeter data showed shaded fruit having redder flesh than chemically-thinned ones ($p < 0.05$). Shade-thinning fruit were larger than fruit from chemical thinning ($p = 0.028$). No differences were found for texture analyser data and anatomical analyses, as well, confirming the sensory data.

*Experiment 2:* Black and red net fruit were larger than white net apples ($p = 0.0042$), and blue and yellow were intermediate, despite average number of fruit per tree and average load per tree being similar for the five treatments (data not shown). No differences for chemical composition were found; for colour, the lowest L* value was found in red net and the highest for white net fruit ($p < 0.001$); the highest a* value was observed for red net and the lowest for yellow and white net apples ($p < 0.001$). Dry matter concentration was higher in red and white net than yellow net apples ($p = 0.014$). Red and black net apples were the least and the white net fruit were the firmest, while the highest acoustic response was from the red net sample (Table 3). White and black net apples had the highest number of cells per volume (small cells tightly packed), while red net fruit showed the lowest number (large cells with more intercellular spaces; Figure 3; $p < 0.001$).

## DISCUSSION AND CONCLUSIONS

In experiment 1, despite the efficacy of thinning was comparable between the two treatments, fruit from shading thinning were found to be bigger than fruit from chemical thinning, in accordance to other authors (Solomakhin and Blanke, 2008; Widmer et al., 2008).

No differences in sensory properties were found between the two treatments, except for green flesh attribute by colorimeter, which should reflect the slight difference in green flesh colour coming from sensory analysis, with shaded fruit being less green than chemically-thinned fruit, even if the green intensity perceived by the panel was extremely low both for shading and chemical treatments. Yellow flesh colour showed higher scores and confirmed that yellow colour is predominant in flesh appearance of ‘Rosy Glow’, but no sensory differences were perceived for such attribute.

The absence of differences in texture analyser and cell anatomy analyses leads to suppose that, despite the difference in timing between chemical and shade application, both thinning methods tested here did not impact differently on the crucial cell division phase of fruit growth. This confirms observations that cell division can occur for several weeks after bloom (Corelli Grappadelli, unpublished). Further proof that this potential remains intact is given by the fact that the shaded trees provided larger fruit at harvest.

The differences found for titratable acidity are not confirmed by sensory analysis, suggesting that such differences are too slight to be perceived by human senses.

In experiment 2, differences were highlighted between the products’ sensory profiles and instrumental analyses confirmed their reliability. L* and a* colorimeter parameters varied between the various net colours. Since a* values are representative of wavelengths from green (negative values) to red (positive values; see Schanda, 2007), L* and a* agreed with the perception by the sensory panel of the yellow flesh colour of red net apples as the most intense, and the lowest in white and yellow net fruit.

The red and white nets varied greatly in texture analyser compression and cell anatomy. Red net apples were larger (average weight = 217.0 g) with the lowest number of cells per volume (i.e., large cells not tightly packed), the lowest yield force, and the highest acoustic response at compression; the white net, on the other hand, gave the smallest fruit (average weight = 195.4 g), with small cells closely packed, the highest yield force, and the lowest acoustic response (Fig. 3). The sound produced during compression is related to the expansion of the liquid subjected to turgor pressure from damaged cells into the surrounding air spaces (Duizer, 2001): the higher the volume of air spaces, the higher the sound. Mann et al. (2005), for example, showed higher crispness scores assigned by a sensory panel to apples with a lower number of cells per unit area of volume. A high turgor pressure in red net apple cells could depend either on a higher assimilation and retention of solutes in the cell vacuole during fruit growth, or reduced conversion to starch of the assimilates downloaded from the phloem. Phloem downloading is affected by modified environmental conditions (Morandi et al., 2011). As a matter of fact, high dry matter concentration was recorded in the red net fruit. If this resulted in higher turgor pressure, it could be the reason for the higher sonic response during compression, since the red net apples were observed as the "noisiest" at the texture analyser measurements. The force required to compress the red net samples was the lowest, in accordance with the lower cell density: Larger cells with a higher amount of air spaces cause a decrease in resistance to compression (Volz et al., 2004), even if other authors observed different behaviours related to different structures. Mann et al. (2005), for example, showed that apples with different cell size and number, measured by microscopy, can have similar response at instrumental compression by texture analyser. However, such results were not confirmed by the sensory perception: apple cultivars with the lowest cell number and highest cell size showed very high scores for firmness and crispness evaluated by a sensory panel, and vice versa for the cultivars having the highest cell number and the lowest cell size (Mann et al., 2005). In our work, as well, very high scores for hardness were awarded by the panel in the sensory evaluation of red net fruit, despite it being the treatment with the lowest number of cells per volume (Table 2; Fig. 3). In their study on microscopic behaviour of different apple varieties under compression and tensile test condition, Alamar et al. (2008) showed interesting findings, useful for interpreting our results. First of all, they found that ‘Braeburn’ apples had higher average cell projected area measured by microscopy images (i.e., fewer intercellular spaces) than ‘Jonagored’ apples. The maximum force measured by compression through an 11 mm probe was also higher for ‘Braeburn’, but the maximum strain at failure measured during a micromechanical compression test was higher in ‘Jonagored’ fruit. Cell reorganisation and a compression of the intercellular spaces do actually happen in response to the compression loading. Thus, a matrix with more intercellular spaces and fewer cells per unit space has a higher leeway to tolerate the compression stress before breaking (Alamar et al., 2008). In our sensory protocol, hardness was evaluated by the panel as the resistance to a slight compression by lateral teeth before flesh tissue breaking; thus, in light of the conclusion above, it is possible to explain why apple fruit with a low number of cells per volume and low performance at instrumental compression tests were evaluated as hard by the sensory panel, as was the case of red net apples (Table 2). From our results red net Fuji apples may have matured more quickly than apples from the other shading treatments. Higher dry matter concentration, yellower flesh (due to an increase in carotenoid content), and a significant increase in perceived sweetness can all be considered characteristics of more ripe fruit (Lakso et al., 1995; Kvikliené et al., 2011; Ampomah-Dwamena et al., 2012).

Light spectrum appears to influence physiological mechanisms linked to cell proliferation during fruit growth, that are reflected in changes in texture properties due to a different number and a different size of cells, and light microclimate also affects the ripening process of the fruit. However, more work is needed to better interpret such mechanisms. Sensory analysis was applied to study the perceivable quality of apples grown under innovative orchard management approaches which aim to increase the sustainability of fruit production by conditioning the orchard light microclimate. In the case of the more ecological thinning practice based on shading, the comparison with chemical thinning showed differences which can be measured by instrumental analyses, but not perceivable by human senses, except for green flesh attribute, even if a very low impact on the sensory profile of ‘Rosy Glow’ apples can be ascribed to such attribute. Thus, from the fruit quality point of view, thinning via shading seems to be a potential alternative to chemical, since it allowed achieving comparable yield and better fruit size of ‘Rosy Glow’ apples without affecting fruit sensory quality. Instrumental and anatomical analyses highlighted differences in physical structure of ‘Fuji’ fruit, developed during fruit growth under different photoselective nets, which correspond to differences in hardness perceived by the sensory panel. Together with sensory differences in flesh colour and sweet taste, such differences suggest changes in the ripening mechanism related to the treatment. Thus, we had useful indication about the possible effect of different light spectra on the eating quality of apples, but further investigation on the fruit growth mechanisms under coloured nets will help to better understand how they play and influence the sensory perception of fruit properties.

**Table 1:** Mean values and ANOVA results for experiment 1 (a) and experiment 2 (b) fruit. Significant differences identified by HSD post-hoc test are shown by different letters.

**a.**

| **Product** | **Weight (g) *** | **SSC** | **% dry matter** | **Titratable acidity[a] **** | **% extractable juice** | **L *** | **a *** | **b** | **Cell packing[b]** |
|---|---|---|---|---|---|---|---|---|---|
| Rosy Glow chemical thinning | 197.9 b | 12.8 | 15.6 | 4.46 a | 48.1 | 76.1 b | -2.3 a | 19.2 | 264.1 |
| Rosy Glow shading thinning | 215.0 a | 12.7 | 16.6 | 5.29 b | 47.2 | 75.5 a | -2.0 b | 19.1 | 258.8 |

**b.**

| **Product** | **Weight (g) *** | **SSC** | **% dry matter *** | **Titratable acidity[a]** | **% extractable juice** | **L **** | **a **** | **b** | **Cell packing[b] **** |
|---|---|---|---|---|---|---|---|---|---|
| Fuji Black net shading | 220.9 a | 11.9 | 13.6 ab | 3.63 | 59.4 | 74.7 a | -2.9 b | 20.1 | 222.6 bc |
| Fuji Blue net shading | 208.2 ab | 11.8 | 13.5 ab | 3.54 | 55.8 | 74.4 ab | -2.8 b | 20.8 | 201.1 ab |
| Fuji Red net shading | 217.0 a | 12.8 | 14.7 a | 3.30 | 57.0 | 74.0 b | -2.0 c | 21.1 | 189.0 a |
| Fuji White net shading | 195.4 b | 12.9 | 14.1 a | 3.56 | 58.9 | 74.8 a | -3.3 a | 20.6 | 234.2 c |
| Fuji Yellow net shading | 203.2 ab | 12.0 | 12.4 b | 3.23 | 55.4 | 74.5 ab | -3.4 a | 20.2 | 204.5 ab |

a: meq malic acid/100 g juice
b: cells/mm$^3$
* = ANOVA *p*-value < 0.05
** = ANOVA *p*-value < 0.001

**Table 2:** Mean values for sensory attributes evaluated by the sensory panel for 'Rosy Glow' apples from experiment 1 (a) and 'Fuji' apples from experiment 2 (b). Significant differences for product factor highlighted by HSD post-hoc tests are shown by different letters.

**a.**

| Product | Green Flesh * | Yellow Flesh | Juiciness | Hardness | Flouriness | Crunchiness | Graininess | Fibrousness | Sweet taste | Sour taste |
|---|---|---|---|---|---|---|---|---|---|---|
| Rosy Glow chemical | 2.6 b | 30.9 | 47.6 | 54.3 | 6.1 | 55.0 | 15.6 | 50.5 | 48.3 | 14.3 |
| Rosy Glow shading | 1.2 a | 29.9 | 46.1 | 53.0 | 7.3 | 55.6 | 12.2 | 55.5 | 49.6 | 17.5 |

**b.**

| Product | Green Flesh | Yellow Flesh ** | Juiciness | Hardness * | Flouriness | Crunchiness | Graininess | Fibrousness | Sweet taste ** | Sour taste |
|---|---|---|---|---|---|---|---|---|---|---|
| Fuji Black net | 5.2 ab | 33.6 ab | 66.5 | 47.5 ab | 7.1 | 57.1 | 17.3 | 37.1 | 50.6 ab | 10.4 |
| Fuji Blue net | 5.7 ab | 33.1 ab | 68.0 | 51.4 b | 7.2 | 61.3 | 18.2 | 36.5 | 41.3 a | 7.5 |
| Fuji Red net | 4.6 a | 42.3 b | 64.4 | 52.0 b | 5.4 | 62.5 | 17.6 | 40.7 | 54.9 b | 9.0 |
| Fuji White net | 7.7 ab | 27.4 a | 61.7 | 52.7 b | 8.1 | 57.3 | 18.5 | 35.4 | 48.3 ab | 11.5 |
| Fuji Yellow net | 10.0 b | 28.5 a | 65.7 | 43.0 a | 5.8 | 62.5 | 17.7 | 35.7 | 42.4 a | 9.5 |

* = ANOVA *p*-value < 0.05
** = ANOVA *p*-value < 0.001

**Table 3:** Mean values for texture analyser parameters showing significant differences between 'Fuji apples' from experiment 2. Different letters indicate significant differences by HSD post-hoc test.

| Product | Yield Force * | Mean Force * | Nr. Acoustic Peaks * | Mean Acoustic Pressure ** | Acoustic Linear Distance * |
|---|---|---|---|---|---|
| Black net | 7.9 ab | 7.7 a | 83.3 ab | 48.4 ab | 6892 ab |
| Blue net | 8.2 ab | 7.9 ab | 80.9 ab | 48.1 a | 6835 ab |
| Red net | 7.1 a | 7.8 ab | 95.4 b | 49.4 b | 7499 b |
| White net | 8.8 b | 8.7 b | 74.9 a | 48.0 a | 6460 a |
| Yellow net | 8.2 ab | 8.6 ab | 76.9 ab | 48.1 a | 6618 a |

* = ANOVA *p*-value < 0.05

** = ANOVA *p*-value < 0.001

**Fig. 1:** Satellite image showing the distribution of the photoselective hail nets on the 'Fuji' orchard located near Ferrara, Italy, from Experiment 2.

**Fig. 2:** GPA bi-plot (Dim.1: 60.14%; Dim.2: 11.43%) showing the sensory space of the apple samples from experiments 1 and 2, in relation to the sensory attributes evaluated by the trained sensory panel.

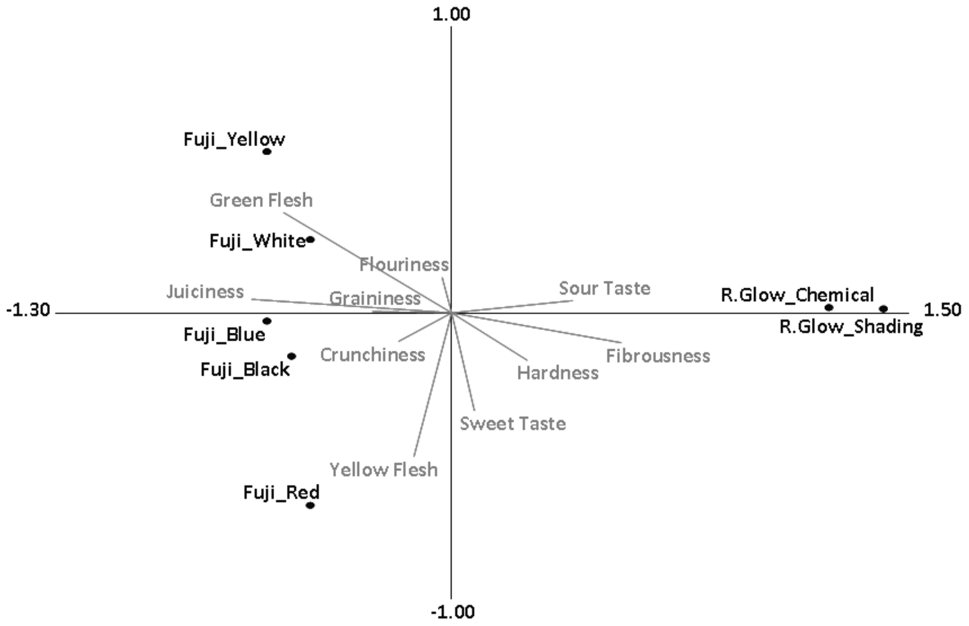
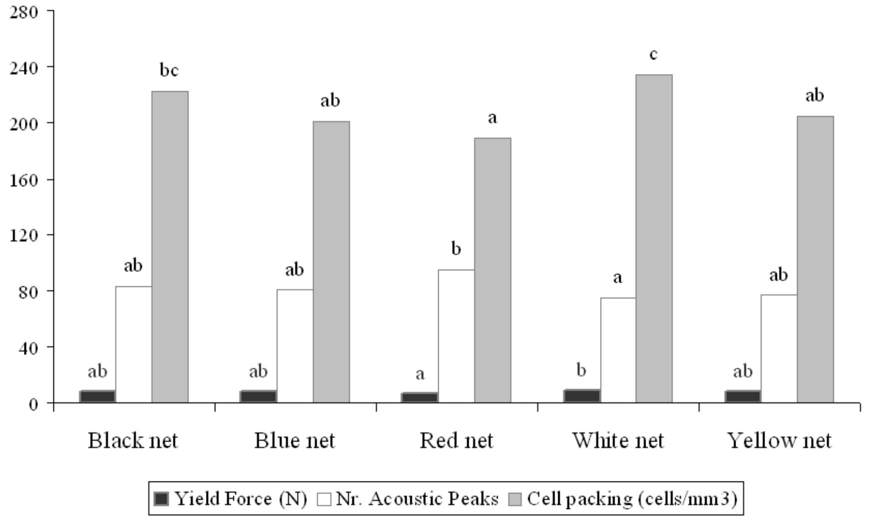


**Fig. 3:** Mean values of yield force (N) and number of acoustic peaks measured by TA-XT texture analyser, and cell packing (cell/mm$^3$) for 'Fuji' apples from experiment 2. Letters show significant differences for each variable as highlighted by HSD post-hoc tests.



# *Chapter VI*

# APPLICATION OF A COMBINED SENSORY-INSTRUMENTAL CHARACTERISATION FOR THE EVALUATION OF PROMISING APPLE ACCESSIONS

## INTRODUCTION

The agronomic and pomological performance of new cultivars must continuously evolve to meet changes in consumer preference and adaptation to climate change.

To date, most breeding efforts have been aimed to improve fruit quality, storage capacity and disease resistance (Chagné et al., 2012; Baldi et al., 2013; Broggini et al., 2013; Costa et al., 2013; Longhi et al., 2013b). In particular for large germplasm collection and breeding materials the evaluation of quality traits is performed by the employment of different instruments, because the very high number of samples to evaluate does not make sensory evaluations suitable for an efficient screening. The implementation of human rather than instruments, made this approach, in general, time-consuming, expensive and not applicable to large samplings. As consequence, fruit from breeding progenies, are normally assessed for their physical and biochemical properties without a complete and exhaustive sensorial characterisation (Tong et al., 1999; Costa et al., 2013; Longhi et al., 2013; Sedov and Serova, 2013). Only in few cases, sensory analyses are applied at a later stage to apple accessions previously selected by instrumental measurements, thus on a reduced number of samples. Selected accessions are then studied in relation to their appeal on consumers or description of their sensory characteristics. The first studies applying sensory science to breeding studies were mainly focused on fruit acceptability measured in relation to flavour and appearance by hedonic and descriptive panels (Redalen, 1988; Granger et al., 1992; Stebbins et al., 1992; Schmitz et al., 2013). Other authors performed fruit evaluation over several years or locations, in order to verify the reliability of their sensory profiles (Kühn and Thybo, 2001; Miller et al., 2005; Paprštein et al., 2006; Donno et al., 2012) However, most of the works showed a lack of details about the applied sensory protocol, or the good practices for sensory analysis appeared not to be followed (Meilgaard et al., 1999; Stone and Sidel, 2004a; 2004b). Recently, Bonany et al. (2013; 2014) studied the acceptability of commercial apple varieties and new genotypes in several countries in Europe, identifying country, age and gender as the most relevant factors affecting consumers' acceptance. Moreover, by preference maps and cluster analysis, the authors defined consumer groups preferring different quality traits in apples, thus providing a useful instrument for marketers and breeders. Hampson et al. (2000) tested the acceptability of new breeding products along several years, to evaluate if the performances were consistent among years, which is an important detail in the evaluation of fruit coming from young trees. Moreover, they demonstrated that a proper sensory profile developed by a trained panel was a better predictor of consumers' appreciation than instruments, determining that analytical measurements are not adequate enough to substitute sensory evaluations in the screening of new breeding materials (Hampson et al., 2000). In fact, if such measures are to accurately predict sensory perception of food properties, human assessment should always be the standard against which instrument readings should be calibrated (Bourne, 2002; Harker et al., 2003).

In this study a new protocol for a descriptive sensory analysis and basic innovative instrumental measurements (Corollaro et al., 2013) was applied in order to perform an effective and reliable sensory profiling of a set of new apple (*Malus* × *domestica* Borkh.) accessions together with their pedigrees. The accessions evaluated in this work derived from the current breeding activities ongoing at the Fondazione Edmund Mach (FEM; San Michele all'Adige, Trento, Italy). The sensory-instrumental tool presented here can be finally proposed as a valuable complement to breeding programs, as it provides information about the real perceivable quality of new selections.

## MATERIALS AND METHODS

### *Plant Materials*

Eight apple selections, together with their seven parental lines, resulting from the FEM breeding program were employed in this investigation (Tables 1a and 1b). For simplicity, the apple accession derived by breeding programs are named as F plus a code from 1 to 8. All trees were located in the same experimental orchard in Mezzolombardo (Trento, Italy). In 2011 and 2013, fruit were picked at commercial maturity and stored for 2 months in a refrigerated cell (A; 2°C, 98% RH, normal atmosphere). In 2012, fruit were instead kept for 180 days in controlled atmosphere (CA; 0.8-0.9% $CO_2$; 1.4-1.6% $O_2$; 1°C; > 90% RH), a condition closer to commercial practice. During this year, the cultivar ‘Cripps Pink’ was not evaluated, due to a heavy scab infection that compromised the entire fruiting. Before the analysis, twenty fruit per genotype were kept at room temperature for 24 h. Samples were then prepared as previously proposed by Corollaro et al. (2013), cutting flesh discs (1.8 cm of diameter and 1.2 cm of thickness) from three slices cut perpendicular to the fruit core which were treated with an antioxidant solution (0.2% citric acid, 0.2% ascorbic acid, 0.5% calcium chloride) in order to prevent flesh browning. Flesh discs from the same apple were used for both sensory and instrument analysis.

### ***Instrumental analysis***

Texture analysis was performed on the apple discs with a TA-XT texture analyser equipped with an acoustic envelop detector device (Stable MicroSystem Ltd., Godalming, UK), following the methodology and the settings described in Costa et al. (2011 and 2012). Nine mechanical and four acoustic parameters were derived on the combined (mechanical and acoustic) profiles (Table 2). Extractable juice (% juice) was also measured in duplicate by weighing the juice squeezed from eight flesh discs/sample (each disc was isolated from a different fruit) and expressed as percentage of fresh weight. Soluble solid content (SSC) and titratable acidity were measured in duplicates on the juice extracted from 12 flesh discs from different fruit for each sample, by using a DBR35 refractometer (XS Instruments, Poncarale, Brescia, Italy) and a Compact Titrator (Crison Instruments S.A., Alella, Barcelona, Spain), respectively. NaOH 0.1N was used for titrating apple juice to pH 8.16, and the results were expressed as malic acid milliequivalents/100g juice.

### ***Sensory analysis***

The sensory panel employed in this work included 14 judges in 2011 (4 males; 10 females), 17 judges in 2012 (6 males; 11 females), and 18 in 2013 (9 males; 9 females), all internal to FEM. Nine judges took part in all three panels, while seven judges participated in two of them. Sensory profiling was performed based on the conventional quantitative descriptive method (Stone and Sidel, 2004a). The sensory lexicon was developed using the consensus method (Murray et al., 2001), composed by attributes related to texture and flavour attributes. Details about the panel selection and training, univocal sensory definitions, evaluation procedures, and reference standards for texture, taste, overall odour and overall flavour attributes are reported in Corollaro et al. (2013). In this study, six attributes for specific odour and retro-nasal flavour sensations were also considered (Table 3). The intensity of each attribute was scored by the panel on a linear scale with three anchored points, at 0 (minimum intensity or absence), 100 (maximum intensity) and 50 (for an intermediate level). The sensory analyses were performed once a week, in a sensory laboratory equipped with twelve individual booths. The samples were presented to each panellist in duplicate, in plastic cups labelled with a three digit code and in randomised balanced order. Collected sensory data, before the description of the sensory profile, were verified for their reliability according to the methods described by Næs et al. (2010).

### *Statistical analysis*

Two-factor ANOVA on instrumental and sensory data, considering season and product factors, was performed. Effects with a *p*-value lower or equal to 0.05 were considered as significant, and post-hoc Honestly Significant Difference (HSD) test was performed to locate existing differences. For visualisation of the product sensory space, Principal Component Analysis (PCA) was performed on sensory odour/flavour and texture data-sets. The statistical analyses were made using the STATISTICA 9.1 software (StatSoft, Inc., U.S.A.).

## RESULTS

### *Product instrument evaluation*

Two-factor ANOVA on instrumental data showed significant differences between the accessions for all texture and chemical parameters, with *p* value lower than 0.001 (exception made for F1-F3, with $p = 0.05$). As regards the year factor, differences were found for 11 out of 15 parameters, with significant interaction between season and accession (Table 4). Significant differences were also found in the case of equal storage conditions.

### *Sensory profile of the apple accessions*

The sensory data from the two experimental years, 2011 and 2013, were initially studied to evaluate differences among apple accessions stored with the same atmosphere. Therefore, a two-factor ANOVA considering season and accession revealed differences due to season for all the odour/flavour attributes ($p < 0.05$), with the exception of overall odour, vanilla odour, sweet taste, vanilla and honey flavours (Table 5). Regarding texture, differences between years were found only for juiciness ($p < 0.01$). All attributes discriminated between the accessions, with the exception of some odours (lemon, grass, vanilla, and honey), and for honey retro-nasal flavour. Significant interaction between season and accession was found for sweet and sour tastes, pear flavour, and for the texture attributes of juiciness, crunchiness, flouriness, and graininess (Table 5).

A PCA was then performed on the sensory data from the three years, considering the texture and odour/flavour data sets separately. For the analysis of texture, the first two PCA components explained 92.2% of sample variability. The samples having higher crunchiness and hardness levels are located in the right part of the plot, while samples having high flouriness and graininess are in the left side. The sample distribution varies between the upper and the lower part of the plot depending on juiciness intensity (Figs. 1a and 1b). The majority of the samples are located on the right side of the plot in Figure 2b, showing very similar profiles in terms of hardness, crunchiness and fibrousness. ‘Golden Delicious’, ‘Gala’ and F7 were scored as high in graininess and flouriness and low in hardness and crunchiness, and the prolonged storage in controlled atmosphere did not guarantee favourable texture features, as the 2012 samples are also located in the left side of the plot. As for the odour and flavour analysis, the first two components explained 58.5% of the total variability of the apple flavour. Odour and aroma attributes are mainly explained by the first principal component (Fig. 2a), with samples characterised by a higher level of odour and aroma on the left side of the space (Fig. 2b). Together with astringency and grass and lemon flavours, sweet and sour taste led to the second principal component, with sweet samples in the upper side and sour apples in the lower part of the PCA space. As highlighted by the dotted shapes on Figure 2b, the samples from 2011 tend to be located in the left part, while the samples from 2012 are all located more towards the right part, showing a poor flavour profile, with very low odour and aroma intensities, with the exception of F3, located in the left side because of a very high sweet taste intensity. For some parental cultivars and new selections, fruit from 2011 and 2013 (stored in normal atmosphere) are closely plotted on the two-dimension PCA space, showing quite similar profiles (see F1; ‘Goldrush’; ‘Cripps Pink’). ‘Gala’ and ‘Fuji’ on the contrary showed a very different sensory characterisation as shown by the multivariate analysis.

To make an effective comparison between the new selections and their parental accessions, spider plots reporting the odour/flavour and the texture sensory profiles from 2011 data-set are shown in Figure 3.

### ***Sensory properties of new selections in relation to parentals***

Since crunchiness and sweet taste are considered two of the most important factors leading to consumer preference in apples (Daillant-Spinnler et al., 1996; Jaeger et al., 1998; Péneau et al., 2006), it is worth to highlight that these two sensory parameters were considered as a good trait in the selection process of the new accession. Good crunchiness performances were shown by F2, F5, F8 (Fig. 4a). F1 showed a slight decrease in crunchiness during the years, while F3 showed a slight reduction in 2013 (not significant), and F4 showed a very good crunchiness performance in both 2011 and 2013, but not after a prolonged CA storage in 2012. Its crunchiness decrease was confirmed by texture analyser measurements, showing a decrease in acoustic response in 2012 samples (data not shown). F7 never showed a good crunchiness level, and F6 showed a large but not significant reduction in 2013, confirmed by a decrease in both mechanical and acoustic parameters from texture analyser measurements. F3 resulted the sweetest accession, exceeding also the value of its parental varieties, 'Fuji' and 'Gala' (Fig. 4b). In the case of F1, F2, F4, F5, F6 the sweetness intensity was generally maintained across years, even in the CA storage condition. In F4 and F6, in particular, sweetness did not appear to be affected by any decrease in crunchiness. Both F7 and F8 showed a slight decrease of sweet taste as years passed. 'Fuji' apples, instead, showed a dramatic decrease in sweet taste in 2012, when CA storage was applied.

## DISCUSSION

The results from two-factor ANOVA on instrumental data showed that apple accessions were different in terms of physical and chemical properties and that the set of different accessions changes every year for almost all the parameters, even in the case of same storage conditions. The two-factor ANOVA on sensory data showed very similar results, confirming that in different years, even under equal storage conditions, the different apple accessions can develop different sensory properties, as already observed for the instrumental data. It is known that the physical and chemical properties of a fruit can change not only because of post-harvest condition, but also for pre-harvest environmental factors. Light intensity, water stress and temperature, for example, can be responsible for the variability observed from one year to another (Sams, 1999; Fellman et al., 2000; Johnston et al., 2002).

The results from the PCA on texture sensory data are in accordance with Allan-Wojtas et al. (2003), who found that a crispness/mealiness vector drove the first principal component in the PCA on their sensory data about apple texture properties. Juiciness and melting attributes were representative of the second principal component. This confirmed that most of the sensory variability in apple was related to the mechanical features of the cell wall and middle lamella. Indeed, the alternative properties of crunchiness and mealiness are determined by the strength proper of the intercellular linkages. When cell bonds are strong, the compression of the structure determines a breaking of the cell wall, corresponding to the detection of a high hardness and crunchiness perception. When cell bonds of the middle lamella are weak, the physical compression produces a sliding of the cells, without disrupting the cell wall, i.e. without the generation of any acoustic emission, typical of the mealy fruit (Harker and Hallet, 1992; Duizer, 2001).

In the plot in Figure 1b, the samples appeared to be spread out on the map in a way that can not be related to the different years. Indeed, from ANOVA on sensory data, most of the texture attributes showed interaction between year and accession factors. This confirmed that each accession followed a different trend in changing texture characteristics from one year to another. No clear and universal trends were observed, even for apples stored in CA, as already confirmed by texture instrument description. To explain the different behaviours, it is important to note that genetic factors have already been indicated as one of the main source of variability in fruit quality traits, along with climatic and environmental factors (Sams, 1999; Fellman et al., 2000). Thus, it is possible to assume that environmental factors modify the expression of different fruit quality traits depending on the different genetic profile. For example, ‘Golden Delicious’ apples showed high juiciness in 2011 but not in 2013. This is in agreement with the instrument measurements of extractable juice, even if no difference in sensory juiciness was measured between the two years. A similar trend was also detected for ‘Red Chief’, F8 and F3 samples. Among the new selections, the highest variability in texture profile during the three years was observed for F6.

As for odour and flavour profile, the distribution of the samples in the score plot in Figure 2b highlights that samples from 2012 had a poor odour and flavour profile. Other authors have already demonstrated that controlled atmosphere is responsible for a decreased volatile release (Mattheis et al., 1998; Echeverria et al., 2008; Lo Scalzo et al., 2003; Lara et al., 2007), due to an inhibition of either gene expression or activity of enzymes controlling esters production (Villatoro et al., 2008).. The 2013 apples instead showed intermediate profiles between the richest 2011 samples and the 2012 fruit, suggesting some variability in terms of odour, aroma and taste related to the different years.

The first two components from the PCAs on odour/flavour and texture profiles gave a general overview about the relation between the new selections and their parental varieties.

Given that differences are shown by the different accessions from one year to another, there are selections showing a quite constant profile, being more similar to one of the two parents. F3, indeed, showed a texture profile similar to ‘Fuji’, but it is far from both ‘Gala’ and ‘Fuji’, for its odour and flavour profile, showing very high sweet taste intensity (Fig. 3c). F1 had a flavour profile closer to ‘Cripps Pink’ than ‘Pinova’ (Fig. 3a). In the case of F8, the texture profile was clearly similar to ‘Red Chief’, and very different from ‘Golden Delicious’ (Fig. 3h).

In other cases, the new selections are completely different from both parents, as it is for F5, which is quite far from both ‘Fuji’ and ‘Pinova’ in all the years considered here (Figs. 1b; 2b).

Other fruit are more difficult to describe and to compare to the parental varieties, because of an inconstant sensory profile. F4 and F6, for example, showed dramatic changes in their texture properties from one year to another. Thus, F4 appeared to be closer to ‘Fuji’ than ‘Cripps Pink’ both for flavour and texture profiles (Fig. 3d), but it was not true in 2012. Maybe such new selection did not tolerate well the prolonged storage in CA. F6 showed a texture profile more similar to ‘Goldrush’ (Fig. 3f), even if not confirmed every year. However, it is important to remember that any difference in sensory properties and instrumental parameters between air and CA storage can not be ascribed to the storage condition only, since in this study the fruit came from three different years. Thus, many other environmental and growing factors might have affected fruit quality before storage, and not all of them are easy to take into account in a multivariate approach.

In other cases, it is possible to describe the new selections as interesting combinations of the two parents’ properties. That is true in the case of F7, which was closer to ‘Pinova’ than ‘Gala’ for its sweet and sour taste intensities, while for texture the situation was the opposite since it was closer to ‘Gala’ (Fig. 3g).

As for the attribute of interest – crunchiness ad sweet taste – no significant differences for crunchiness were found for any accession among the three years, confirming that no differences in terms of acoustic properties were perceivable (Fig. 4a). Crunchiness is really important in defining fruit freshness and it is among the attributes leading consumer preferences for apples (Péneau et al., 2006; Harker et al., 2008). However, the strong differences found in terms of overall texture profile (Fig. 1b) suggest that it can not be considered the only factor to evaluate the new accession texture performances, which are the result of a combination of mechanical and acoustic properties all influencing each other. This demonstrates that a complete and detailed sensory profile is necessary to have an overview of the new accession properties and to study what consumers would really perceive.

Moreover, in the case of sweet taste (Fig. 4b), we found no correspondence between SSC and sweet taste perception in the case of Fuji apples, which showed a strong decrease in sweetness in 2012. This result confirmed that soluble solid concentration alone is not a valid predictor of sweetness, since its perception is strongly influenced by other sensory properties, as previously observed by other authors (Harker et al., 2002b; Echeverría et al., 2008).

## CONCLUSIONS

The sensory-instrumental tool here presented showed to be effective in the description of new apple accessions, providing a complete definition of their sensory profile, which was confirmed by instrumental measurements. The method highlighted differences and similarities between the accessions, defining the potential peculiarities that some of the FEM selections showed with regards to their parents, with some of them appearing to be new combinations of the two parental sensory profiles. In some cases they showed very good performances, even after prolonged storage in controlled atmosphere, providing preliminary information about their storability and their suitability for real market conditions.

The proposed methodology represents a valuable approach for the description of novel accessions throughout seasons and different storage conditions. Moreover, the sensory description will represent an inestimable indication of the potential that new apple genotypes have in meeting consumer preferences.

**Table 1:** Apple samples analysed: breeding selections (a) and commercial genotypes (b). In “Code” column the codes used in plots in Figs. 1, 2 and 3 are reported. In “Storage condition” column, “A” is for 2 months in air; “CA” is for 180 days in controlled atmosphere. Weight is expressed as mean value on 20 fruit; SSC and titratable acidity are measured on 12 fruit; % juice is measured on 8 fruit.

**a.**

| **Breeding selections** | **Code** | **Season** | **Harvest** | **Storage condition** | **Weight (g)** | **SSC** | **Titratable acidity[a]** | **% juice** |
|---|---|---|---|---|---|---|---|---|
| FEM selection 1 (Pinova x Cripps Pink) | F1 | 2011 | 27/10/2011 | A | 251,2 | 15,9 | 10,3 | 44,0 |
| | | 2012 | 22/10/2012 | CA | 251,7 | 14,8 | 9,7 | 27,6 |
| | | 2013 | 24/10/2013 | A | 196,1 | 13,5 | 8,6 | n.d. |
| FEM selection 2 (Goldrush x Pinova) | F2 | 2011 | 29/09/2011 | A | 235,8 | 14,5 | 10,2 | 45,1 |
| | | 2012 | 28/09/2012 | CA | 217,3 | 14,3 | 10,0 | 28,9 |
| | | 2013 | 03/10/2013 | A | 216,4 | 12,9 | 10,5 | 11,1 |
| FEM selection 3 (Fuji standard x Galaxy) | F3 | 2011 | 08/09/2011 | A | 183,1 | 14,7 | 4,5 | 38,2 |
| | | 2012 | 06/09/2012 | CA | 192,8 | 15,7 | 4,5 | 26,3 |
| | | 2013 | 19/09/2013 | A | 149,5 | 14,5 | 5,8 | 35,4 |
| FEM selection 4 (Fuji standard x Cripps Pink) | F4 | 2011 | 18/10/2011 | A | 240,5 | 14,2 | 3,6 | 50,6 |
| | | 2012 | 18/10/2012 | CA | 257,3 | 13,5 | 3,2 | 28,9 |
| | | 2013 | 22/10/2013 | A | 204,5 | 13,5 | 5,6 | 24,7 |
| FEM selection 5 (Fuji standard x Pinova) | F5 | 2011 | 27/10/2011 | A | 237,5 | 18,2 | 7,3 | 40,1 |
| | | 2012 | 25/10/2012 | CA | 243,7 | 15,5 | 5,4 | 23,4 |
| | | 2013 | 28/10/2013 | A | 230,5 | 15,5 | 7,7 | 15,9 |
| FEM selection 6 (Royal Gala x Goldrush) | F6 | 2011 | 20/10/2011 | A | 256,5 | 15,2 | 8,8 | 43,4 |
| | | 2012 | 22/10/2012 | CA | 193,4 | 14,0 | 7,5 | 15,9 |
| | | 2013 | 28/10/2013 | A | 200,4 | 13,5 | 8,1 | 13,8 |
| FEM selection 7 (Royal Gala x Pinova) | F7 | 2011 | 18/08/2011 | A | 198,9 | 11,1 | 5,7 | 42,1 |
| | | 2012 | 23/08/2012 | CA | 193,6 | 11,8 | 6,3 | 29,0 |
| | | 2013 | 29/08/2013 | A | 180,7 | 13,9 | 9,7 | 47,9 |
| FEM selection 8 (Golden Delicious x Scarlet Spur) | F8 | 2011 | 15/09/2011 | A | 233,3 | 14,2 | 3,2 | 44,2 |
| | | 2012 | 17/09/2012 | CA | 198,1 | 11,4 | 3,4 | 32,0 |
| | | 2013 | 23/09/2013 | A | 221,0 | 12,9 | 6,7 | 42,9 |

**b.**

| **Commercial genotypes** | **Code** | **Season** | **Harvest** | **Storage condition** | **Weight (g)** | **SSC** | **Titratable acidity**[a] | **% juice** |
|---|---|---|---|---|---|---|---|---|
| Cripps Pink | PIN | 2011 | 24/10/2011 | A | 209,3 | 14,4 | 5,9 | 38,9 |
| | | 2013 | 04/11/2013 | A | 182,8 | 12,1 | 7,9 | 18,8 |
| Fuji (Kiku 8) | FUJ | 2011 | 06/10/2011 | A | 270,0 | 13,7 | 3,5 | 45,2 |
| | | 2012 | 18/10/2012 | CA | 251,6 | 13,0 | 3,3 | 27,5 |
| | | 2013 | 17/10/2013 | A | 228,0 | 14,1 | 5,1 | 22,0 |
| Gala (Schniga) | GAL | 2011 | 09/08/2011 | A | 185,7 | 10,9 | 4,4 | 39,8 |
| | | 2012 | 16/08/2012 | CA | 197,1 | 11,2 | 3,6 | 37,7 |
| | | 2013 | 26/08/2013 | A | 170,1 | 13,0 | 3,9 | 38,8 |
| Golden Delicious (B) | GOL | 2011 | 12/09/2011 | A | 255,1 | 11,8 | 3,9 | 51,5 |
| | | 2012 | 12/09/2012 | CA | 251,2 | 12,8 | 4,2 | 29,2 |
| | | 2013 | 16/09/2013 | A | 222,1 | 12,6 | 5,0 | 21,9 |
| Goldrush | GDR | 2011 | 24/10/2011 | A | 280,7 | 14,5 | 8,8 | 52,7 |
| | | 2012 | 25/10/2012 | CA | 297,5 | 13,4 | 8,2 | 20,6 |
| | | 2013 | 28/10/2013 | A | 245,4 | 12,5 | 8,3 | 20,9 |
| Pinova | PNV | 2011 | 13/09/2011 | A | 231,7 | 12,7 | 5,7 | 46,6 |
| | | 2012 | 17/09/2012 | CA | 215,7 | 11,8 | 4,7 | 40,1 |
| | | 2013 | 19/09/2013 | A | 194,0 | 12,2 | 7,7 | 41,2 |
| Red Chief | RCF | 2011 | 31/08/2011 | A | 299,3 | 11,2 | 4,1 | 50,6 |
| | | 2012 | 30/08/2012 | CA | 246,3 | 11,2 | 4,1 | 38,7 |
| | | 2013 | 09/09/2013 | A | 222,9 | 13,3 | 6,0 | 42,2 |

**a**: meq malic acid/100g juice

**Table 2:** Mechanical and acoustic parameters provided by texture analyser measurements. In “Code” column, the codes used in Table 4 are reported.

| Category | Code | Description |
|---|---|---|
| Mechanical | F1 | Yield Force |
| Mechanical | F2 | Max Force |
| Mechanical | F3 | Final Force |
| Mechanical | FP | N° Force Peaks |
| Mechanical | A | Area |
| Mechanical | FLD | Force Linear Distance |
| Mechanical | Y | Young's Module |
| Mechanical | F4 | Mean Force |
| Mechanical | F1-F3 | Delta Force |
| Acoustic | AUXP | N° Acoustic Peaks |
| Acoustic | AUX1 | Max Acoustic Pressure |
| Acoustic | AUX2 | Mean Acoustic Pressure |
| Acoustic | AUXLD | Acoustic Linear Distance |

**Table 3:** Sensory vocabulary used by the trained panel.

| Category | Descriptor | Definition |
|---|---|---|
| Texture | Hardness | Resistance of the sample at the first chews with molars |
| Texture | Juiciness | Amount of juice released during chewing (first three chews) |
| Texture | Crunchiness | Sound (pitch/intensity) produced by the sample during 5 molar chews |
| Texture | Flouriness | Degree of flesh breaking in small and dry fragments/granules during chewing |
| Texture | Fibrousness | Degree of flesh breaking during chewing in thick and fibrous fragments/granules |
| Texture | Graininess | Numbers/size of fragments/granules produced during chewing |
| Odour & Flavour | Sweet taste | Sweet taste sensation |
| Odour & Flavour | Sour taste | Sour taste sensation |
| Odour & Flavour | Astringency | Tactile dryness sensation in the mouth (at the end of mastication) |
| Odour & Flavour | Overall Odour | Overall odour sensation |
| Odour & Flavour | Overall Flavour | Overall flavour sensation |
| Odour & Flavour | Pear | Specific odour (Od) or retro-nasal flavour (Fl) sensation |
| Odour & Flavour | Banana | Specific odour (Od) or retro-nasal flavour (Fl) sensation |
| Odour & Flavour | Lemon | Specific odour (Od) or retro-nasal flavour (Fl) sensation |
| Odour & Flavour | Grass | Specific odour (Od) or retro-nasal flavour (Fl) sensation |
| Odour & Flavour | Vanilla | Specific odour (Od) or retro-nasal flavour (Fl) sensation |
| Odour & Flavour | Honey | Specific odour (Od) or retro-nasal flavour (Fl) sensation |

**Table 4:** *p*-values from factorial ANOVA on instrumental data, performed considering season and product factors. *P*-values lower than 0.05 were considered significant. Specific definitions of coding for texture analyser parameters are reported in Table 2.

| Parameter | Season | Product | Season*Product |
|---|---|---|---|
| F1 | 0.211 | 0.000 | 0.000 |
| F2 | 0.008 | 0.000 | 0.000 |
| F3 | 0.730 | 0.000 | 0.000 |
| FP | 0.000 | 0.000 | 0.000 |
| A | 0.001 | 0.000 | 0.000 |
| FLD | 0.000 | 0.000 | 0.000 |
| Y | 0.000 | 0.000 | 0.000 |
| F4 | 0.002 | 0.000 | 0.000 |
| F1-F3 | 0.684 | 0.005 | 0.095 |
| AUXP | 0.000 | 0.000 | 0.000 |
| AUX1 | 0.092 | 0.000 | 0.000 |
| AUX2 | 0.000 | 0.000 | 0.000 |
| AUXLD | 0.000 | 0.000 | 0.000 |
| % juice | 0.000 | 0.000 | 0.000 |
| SSC | 0.002 | 0.000 | 0.000 |
| Titratable acidity | 0.000 | 0.000 | 0.000 |

**Table 5:** *p*-values from two-factor ANOVA on sensory data from 2011 and 2013 years, performed considering season and product factors. *P*-values lower or equal to 0.05 were considered significant.

| Attribute | Season | Product | Season*Product |
|---|---|---|---|
| Overall Odour | 0.418 | 0.000 | 0.052 |
| Od-Pear | 0.001 | 0.016 | 0.202 |
| Od-Banana | 0.000 | 0.018 | 0.705 |
| Od-Lemon | 0.025 | 0.653 | 0.958 |
| Od-Grass | 0.000 | 0.148 | 0.289 |
| Od-Vanilla | 0.099 | 0.148 | 0.840 |
| Od-Honey | 0.000 | 0.309 | 0.984 |
| Hardness | 0.774 | 0.000 | 0.175 |
| Juiciness | 0.002 | 0.000 | 0.025 |
| Crunchiness | 0.881 | 0.000 | 0.006 |
| Flouriness | 0.250 | 0.000 | 0.000 |
| Fibrousness | 0.345 | 0.000 | 0.109 |
| Graininess | 0.979 | 0.000 | 0.025 |
| Sweet Taste | 0.461 | 0.000 | 0.009 |
| Sour Taste | 0.000 | 0.000 | 0.004 |
| Astringency | 0.002 | 0.000 | 0.837 |
| Overall Flavour | 0.003 | 0.000 | 0.499 |
| Fl-Pear | 0.000 | 0.014 | 0.021 |
| Fl-Banana | 0.043 | 0.017 | 0.309 |
| Fl-Lemon | 0.000 | 0.000 | 0.221 |
| Fl-Grass | 0.005 | 0.000 | 0.179 |
| Fl-Vanilla | 0.677 | 0.018 | 0.185 |
| Fl-Honey | 0.222 | 0.672 | 0.995 |

**Fig. 1:** Loading (a) and score (b) plots from PCA performed on texture sensory data on the samples from the three years. In plot b, samples from 2011 are indicated by a circle marker; samples from 2012 by a triangle marker; samples from 2013 by a square marker. The new selections are represented by full markers, while parental varieties by empty markers.

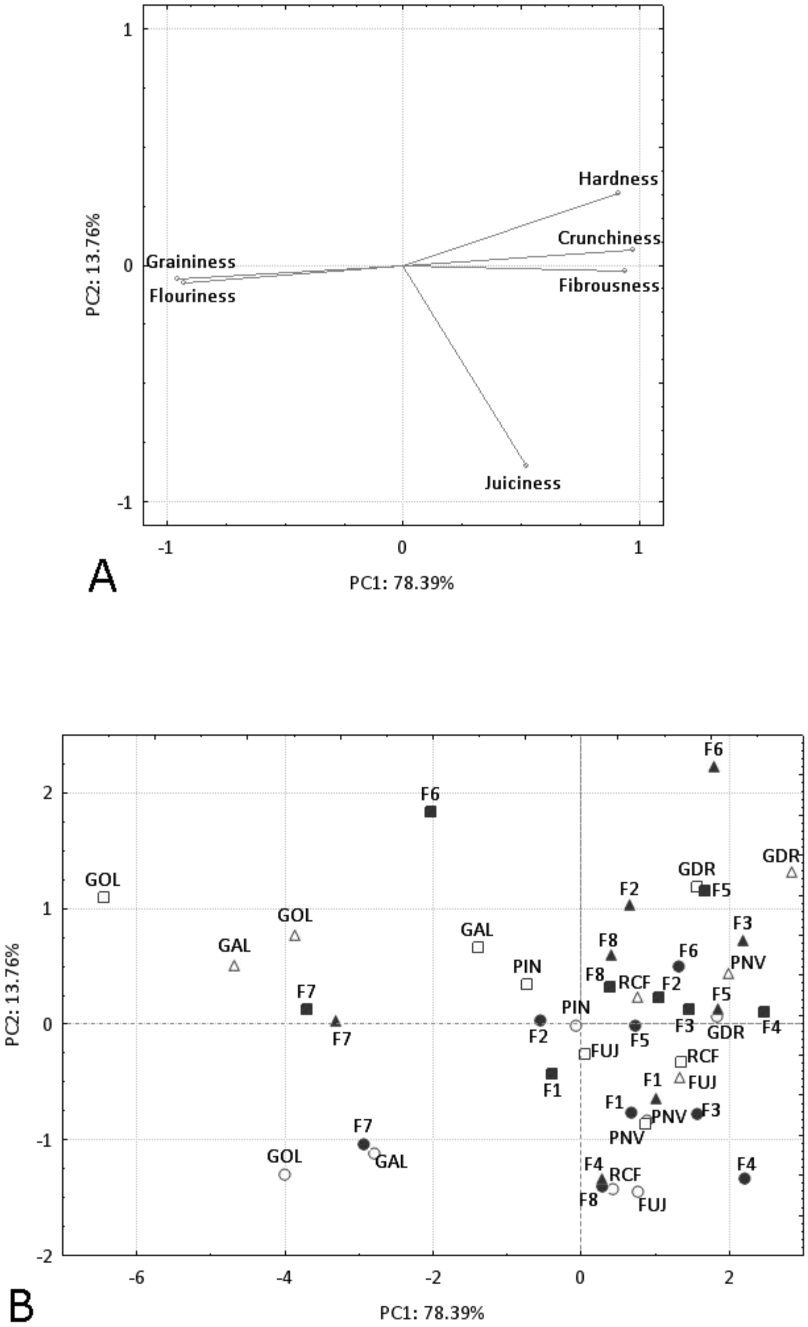


**Fig. 2:** Correlation loading plot (a) and score plot (b) from PCA performed on flavour sensory data on the samples from the three years. In plot b, samples from 2011 are indicated by a circle marker; samples from 2012 by a triangle marker; samples from 2013 by a square marker. The new selections are represented by full markers, while parental varieties by empty markers. The dotted shapes distinguished the samples collected over the three years, 2011 (left), 2013 (middle) and 2012 (right).

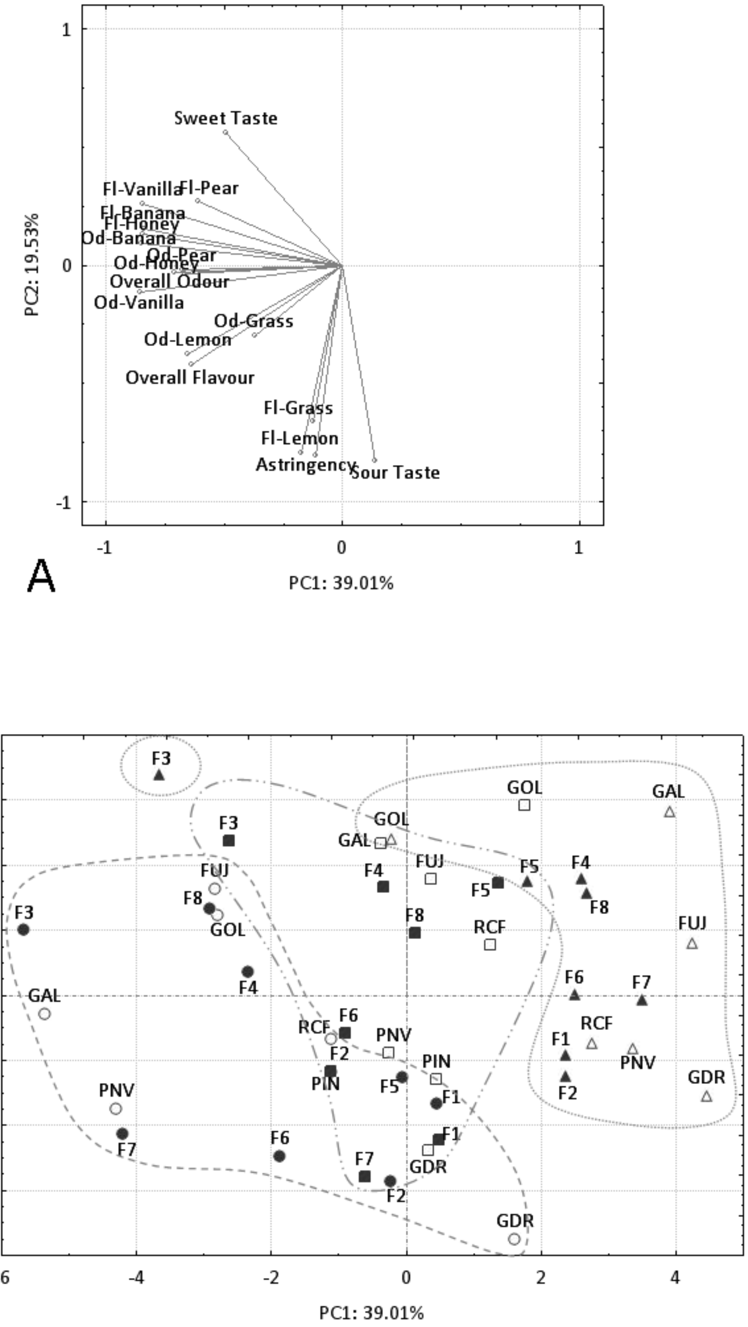
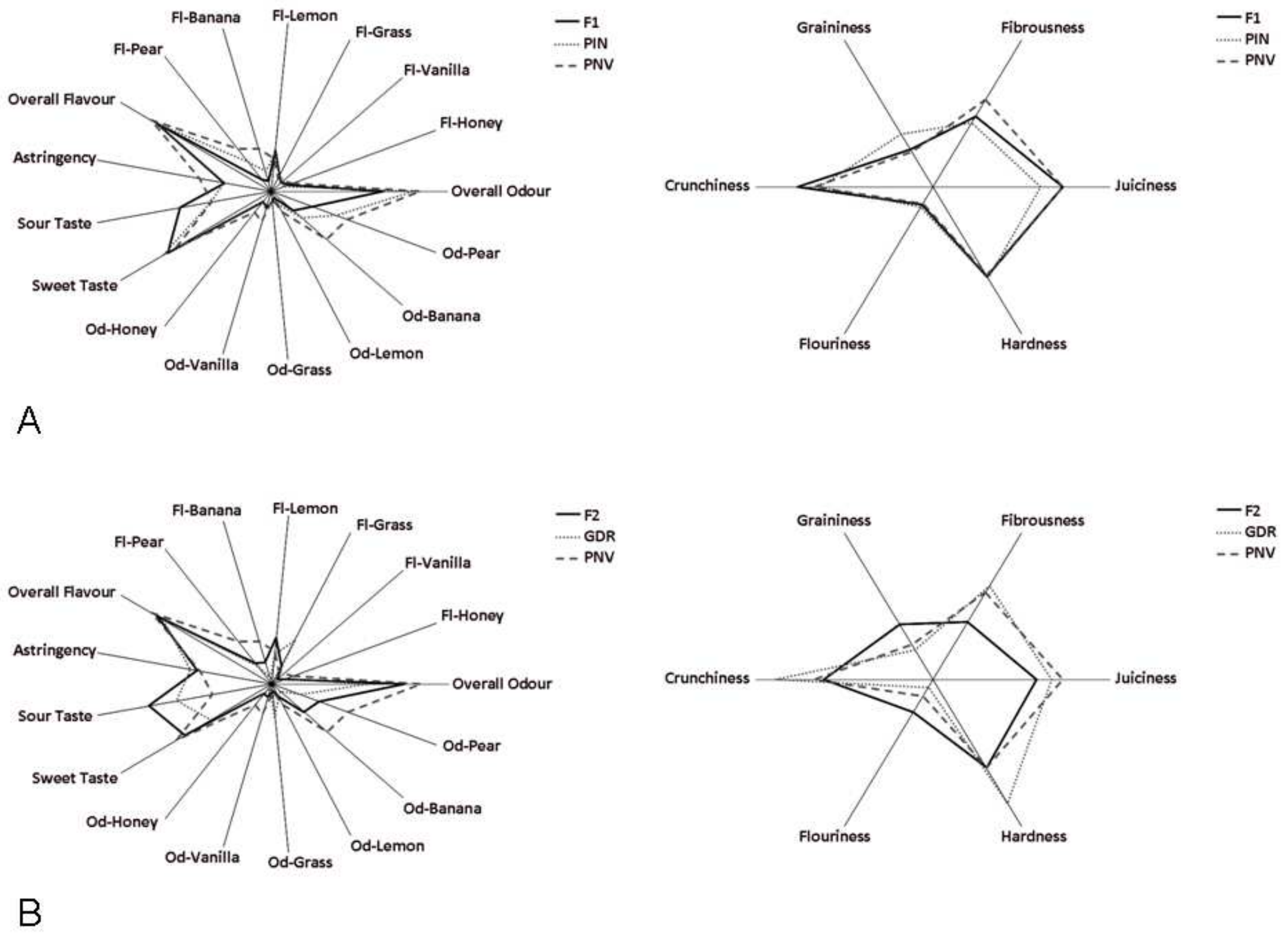
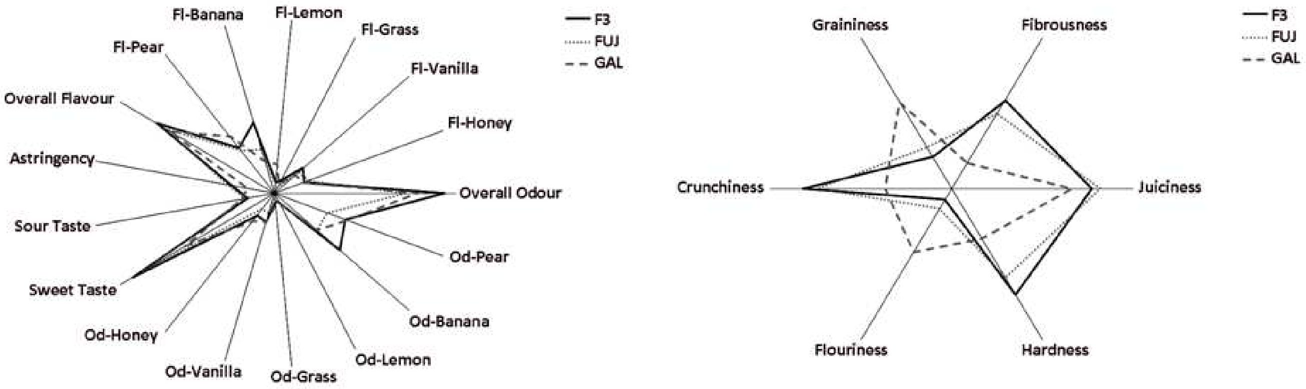


**Fig. 3:** Spider plots showing the odour/flavour and texture sensory profiles of each new selection compared to its parental genotypes.

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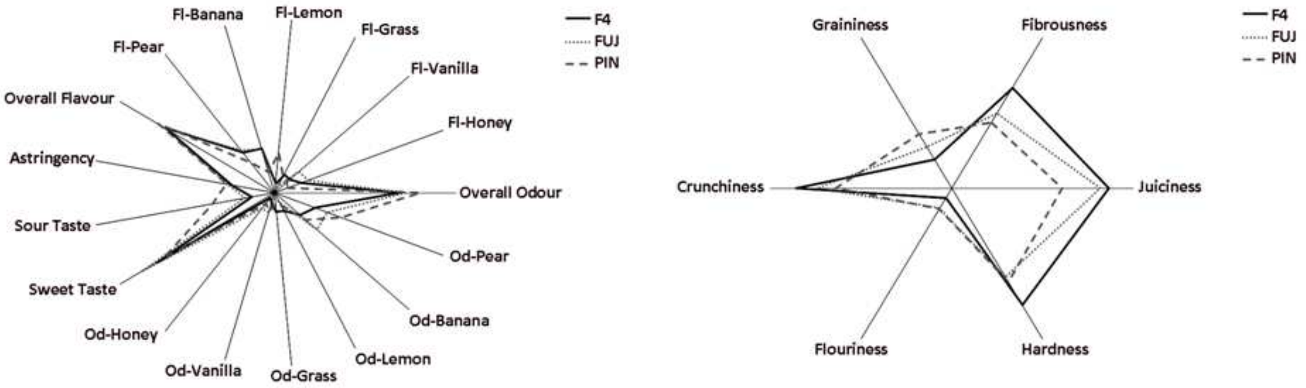
Fl-Lemon
Fl-Grass
Fl-Vanilla
Fl-Honey
Overall Odour
Od-Pear
Od-Banana
Od-Lemon
Od-Grass
Od-Vanilla
Od-Honey
Sweet Taste
Sour Taste
Astringency
Overall Flavour
Fl-Pear
F3
FUJ
GAL
Graininess
Fibrousness
Juiciness
Hardness
Flouriness
Crunchiness
F3
FUJ
GAL

C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Fl-Honey
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Od-Pear
Od-Banana
Od-Lemon
Od-Grass
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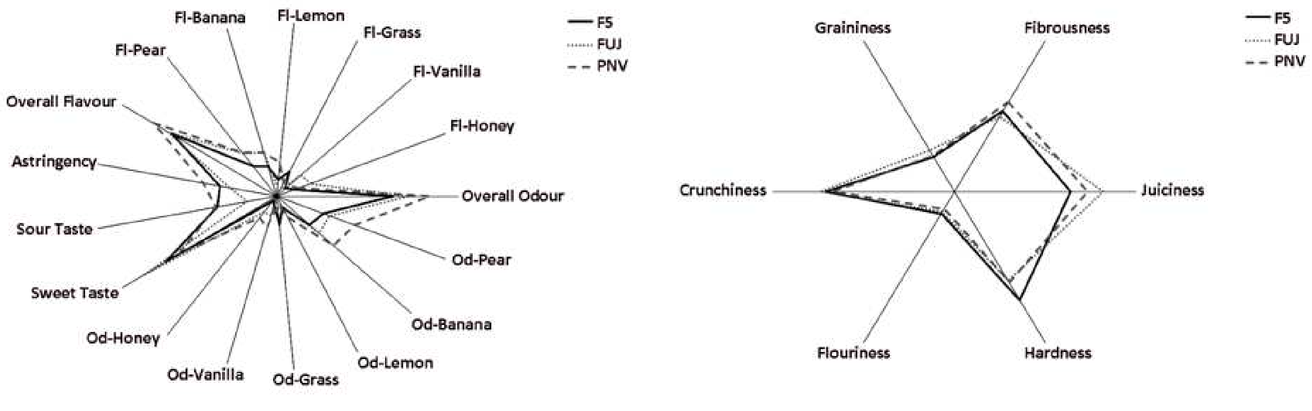
Od-Honey
Sweet Taste
Sour Taste
Astringency
Overall Flavour
Fl-Pear
F4
FUJ
PIN
Graininess
Fibrousness
Juiciness
Hardness
Flouriness
Crunchiness
F4
FUJ
PIN

D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Od-Honey
Sweet Taste
Sour Taste
Astringency
Overall Flavour
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PNV
Graininess
Fibrousness
Juiciness
Hardness
Flouriness
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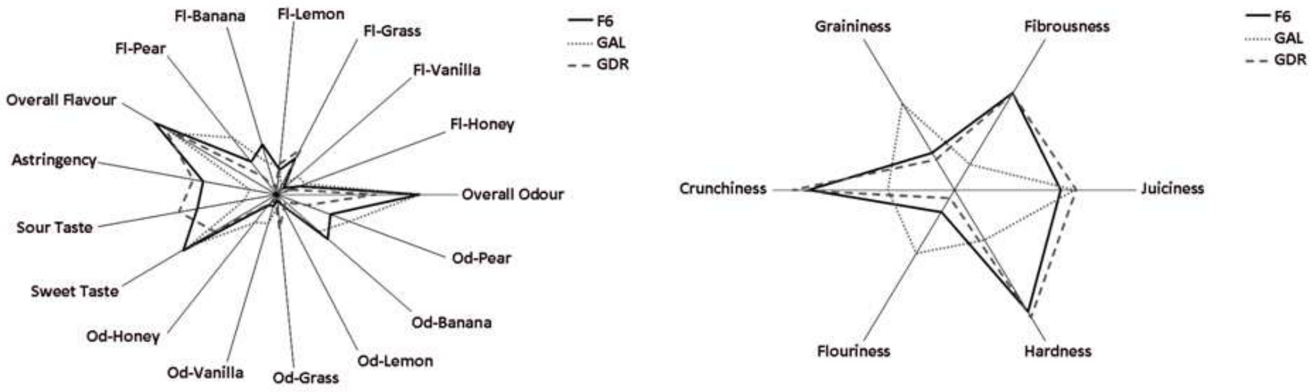
F5
FUJ
PNV

E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Od-Lemon
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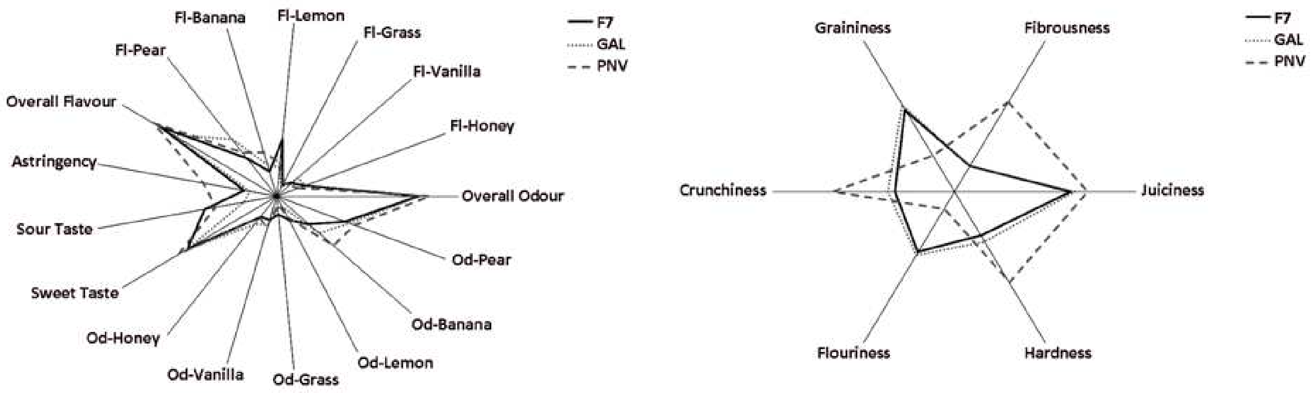
Od-Honey
Sweet Taste
Sour Taste
Astringency
Overall Flavour
Fl-Pear
F6
GAL
GDR
Graininess
Fibrousness
Juiciness
Hardness
Flouriness
Crunchiness
F6
GAL
GDR

F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Od-Lemon
Od-Grass
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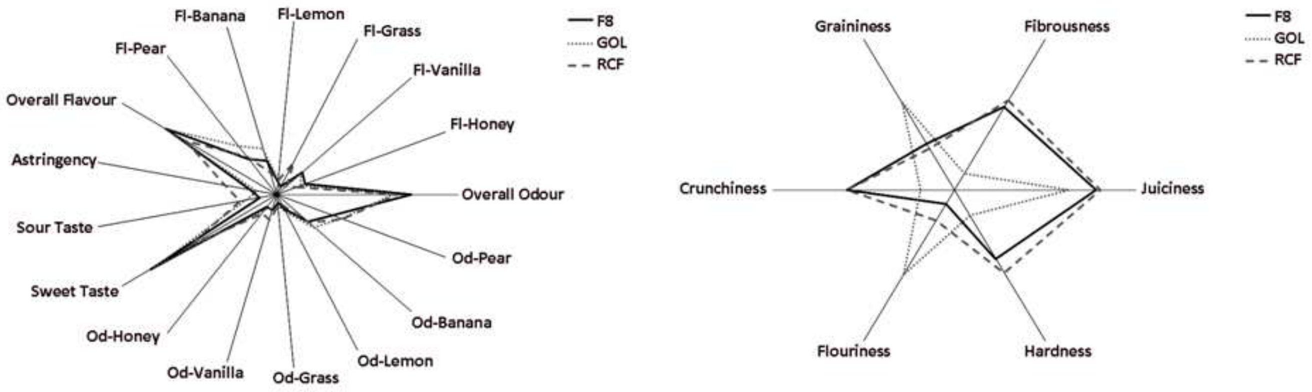
Od-Honey
Sweet Taste
Sour Taste
Astringency
Overall Flavour
Fl-Pear
F7
GAL
PNV
Graininess
Fibrousness
Juiciness
Hardness
Flouriness
Crunchiness
F7
GAL
PNV

G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Od-Banana
Od-Lemon
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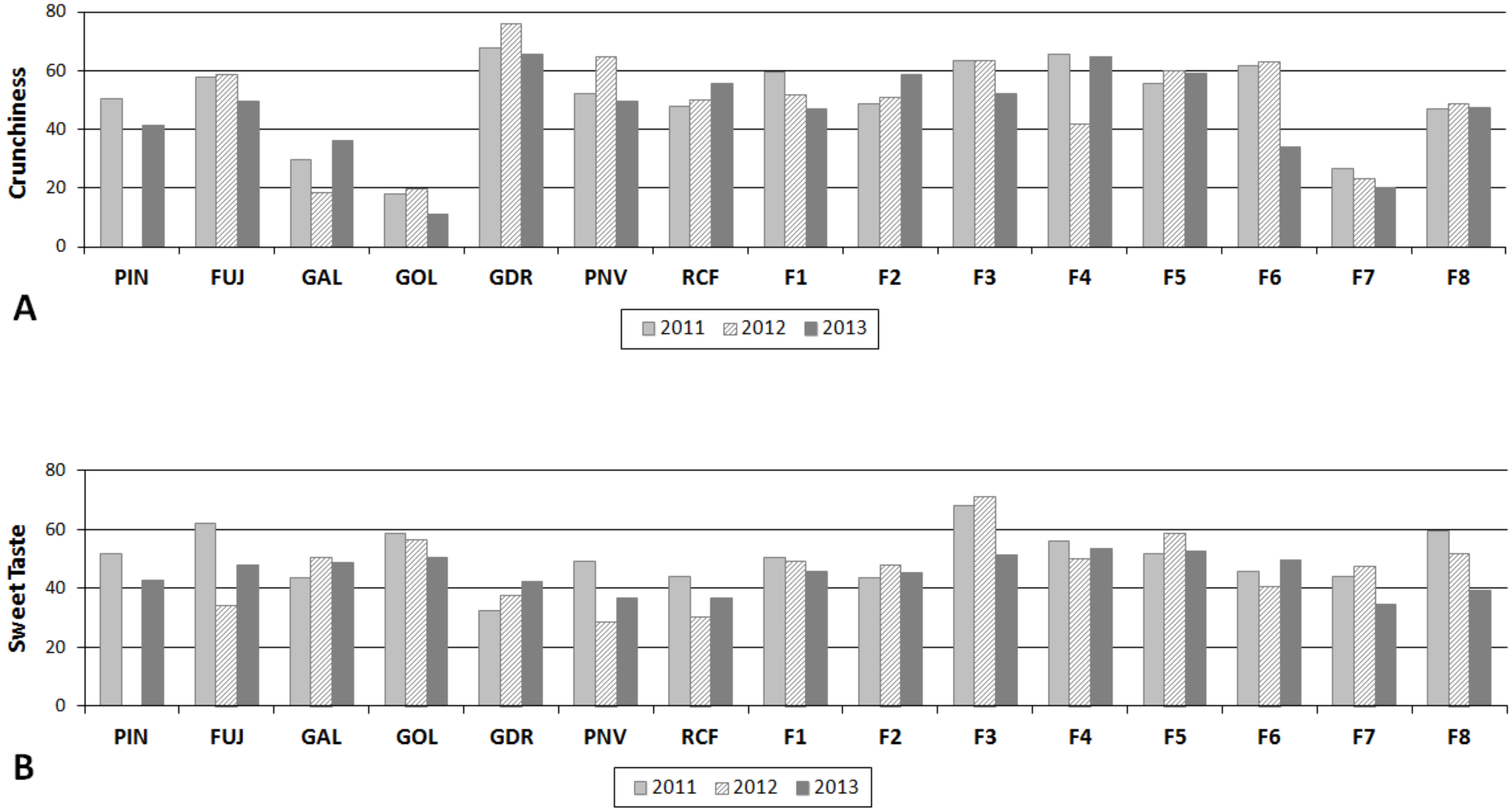
Od-Honey
Sweet Taste
Sour Taste
Astringency
Overall Flavour
Fl-Pear
F8
GOL
RCF
Graininess
Fibrousness
Juiciness
Hardness
Flouriness
Crunchiness
F8
GOL
RCF

H

**Fig. 4:** Crunchiness (a) and sweet taste (b) mean values from sensory analyses on the different apple cultivars and new selections in 2011, 2012 and 2013 years.



# GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

This project was developed with the aim to help all the stakeholders involved in apple production and apple marketing, in order to provide useful and reliable information about sensory perception of fruit quality.

The sensory method was developed following rigorous sensory science criteria, by a proper panel training and panel performance evaluation, to validate the collected sensory data.

The sample preparation procedure here applied was chosen to standardize the evaluation procedures and to ensure that any judge could have a homogeneous sample composed of fruit from the entire batch, rather than a single fruit. Moreover, the sample preparation procedure ensured that the fruit provided to the sensory panel was also subjected to the instrumental analyses. This made the data from sensory and instrumental analyses be really comparable between each other.

The correlation between sensory and instrumental analyses demonstrated that the instrumental measurements here proposed were effective in providing enough information to predict the most important sensory properties of apples. It would be therefore possible to have a complete product sensory profile starting from instrumental data only. In fact, since sensory analysis is time-consuming, expensive, and can not be applied on wide samplings, the final aim is the proposal of our sensory-instrumental tool as a valid source of information to reliably predict apple sensory quality. Therefore, the future perspective is to use a limited number of samples to develop the prediction models by means of a sensory-instrumental data-matrix. The predictive models would be then applied on a wider set of samples subjected to instrumental measures only.

The developed method was also applied on apples subjected to very different examples of pre- and post-harvest factors affecting fruit quality. Such applications demonstrated that the method is able to highlight perceivable differences in apple quality developed at different stages of fruit production chain.

The data collected on apple sensory quality should be then correlated with consumer preference data, to help interpreting which properties are responsible for apple preference or rejection. The combination of sensory, instrumental and consumer preference data will provide the apple producers a general overview, in order to: 1) lead breeding activities toward fruit that better can match consumer expectations, by selection of genotypes carrying specific texture and flavour profiles; 2) help the development of innovative growing practices and post-harvest treatments, to make innovations go hand in hand with the best fruit quality.

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# RINGRAZIAMENTI

Come si fa a riassumere in qualche pagina tutta la gratitudine che ho coltivato in quattro lunghi anni, nei confronti di tutte le persone che in qualche modo hanno contribuito al lavoro che ho svolto, che mi hanno insegnato qualcosa, e che hanno preso parte alla vita che girava intorno a quel lavoro...

Mi sa che sarà una cosa lungha.

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