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# APPLICATION OF A SENSORY-INSTRUMENTAL TOOL TO STUDY APPLE TEXTURE CHARACTERISTICS SHAPED BY ALTITUDE AND TIME OF HARVEST

**Running title: Effect of altitude and time of harvest on apple texture properties**

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## ABSTRACT

BACKGROUND: Texture is important in the preferences of apple consumers. Of the pre-harvest factors affecting fruit quality and especially texture, altitude and subsequent climatic conditions are crucial, determining differences in the physiological mechanisms of fruit growth, ripening stage and chemical composition, as demonstrated by several studies. This work applies a detailed sensory-

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instrumental protocol developed in a previous paper[1] to investigate the impact of altitude, time of harvest and their cross-effect on sensory characteristics of apple, with a focus on texture.

RESULTS: Sensory differences were found in relation to altitude, although the profile results were mainly affected by the time of harvest. Fruit from lower altitude was described as juicier, crunchier and sweeter than samples from higher altitude, which were floury, sourer and more astringent. Texture performance, soluble solids content and titratable acidity corroborated this sensory description. Moreover, anatomical data showed that fruit from lower altitude had a larger volume, a higher number of cells and a higher percentage of intercellular spaces.

CONCLUSION: We demonstrated that differences between fruit from various altitudes can be perceived through human senses, and that the proposed sensory-instrumental tool can be used to describe such differences. This study brings more understanding about the impact of altitude and time of harvest on apple sensory properties. This work could support apple producers, from semi-mountainous regions (Alpes, Tyrol, etc.), in advertising and valorising their products with their specific characteristics in a more efficient manner.

**KEYWORDS**

apple, texture, sensory, altitude, time of harvest.

**INTRODUCTION**

Texture is an essential driver in terms of preference for some apple consumers, according to preferences studies carried out in the apple sector in the last 20 years. Daillant-Spinnler and co-authors[2] set up a preference study on 12 apple varieties from the southern hemisphere. They described the first preference dimension as correlated with juiciness, crispness, hardness and fresh flavour for peeled and unpeeled samples. Jaeger and colleagues[3] considered fresh and aged apples to study consumer preferences. The authors showed that in the first segment, preferences appeared to be driven mainly by textural factors, such as crispness, hardness and juiciness, while in the second one, flavour was the most important characteristic. Another work worth mentioning is a preference study which took into account the full sensory variability that can be found in apples.[4, 5] In this case, they used 28 different apple varieties in order to build more accurate preference models. Once again, they demonstrated that texture attributes were very important in preference models. Crunchiness and juiciness were positively related to preferences. This was confirmed in the more recent work by Bonany and colleagues,[6] who carried out a preference study on eleven varieties in seven European countries. They built a preference map with three main dimensions, of which two involved texture attributes: the second was represented by acidity and firmness, while the third regarded juiciness and crispness. Furthermore, the role of texture has been supported by the existence of a relationship between the firmness/hardness of a product and its acceptability.[7, 8] On the contrary, it has been observed that a mealy/floury, granular or fondant texture in apples was considered as negative and rejected by most consumers.[3, 4]

Despite its significance, texture is not easy to define. It was described by Bourne[9] as the physical properties which arise from the microstructure of the food as perceived by the sense of touch. These properties are related to the deformation, disintegration and flow of the food under the application of a force, and are measured objectively by the functions of force, time and distance. Moreover, Bourne states that textural properties include mechanical (hardness, chewiness and viscosity), geometrical (particle size and shape) and chemical (moisture and fat content) characteristics. In ISO Standards 5492,[10] texture was defined as being perceived through kinaesthesis and somesthesis receptors and (where appropriate) visual and auditory receptors. Some recent works have confirmed that sound information plays an important role in perception of texture properties, such as crunchiness, crispness and more surprisingly hardness.[11, 12] Apple texture is relatively complex and can be described with numerous attributes such as those just mentioned, and also by the following: juiciness/moistness, fibrousness, flouriness/mealiness/starchiness, graininess, fondant, mushiness, pulpy/fluffy, spongy, cohesiveness.[2, 4, 13-16]

Many factors can influence final fruit quality and particularly texture. These factors may have an impact at different stages in the apple production chain: before harvest, at harvest time or after harvesting (respectively called pre-/at-/post-harvest factors). In the first case, they correspond to environmental, cultivation, physiological or genetic factors. In the second, they mainly refer to the type of harvest, the maturity at harvest and fruit size. In the third and last case, they indicate preservation factors (temperature, humidity, controlled/modified atmosphere, duration) and handling processes.[17-19]

Of the pre-harvest factors affecting fruit quality, altitude is one of the most important, as it changes the quality and quantity of light, temperature, hygrometry and day/night variation.[20] Consequently, altitude leads to differences in the physiological mechanisms of fruit growth, ripening stage and chemical composition, as demonstrated by several studies.[21-25] All the factors above are related to final fruit texture and flavour characteristics. However, to our knowledge, only a few studies have applied sensory analysis to evaluate real perceptible differences between apples grown at different altitudes. Eccher and colleagues[26] described the differences existing between apples grown at 3 and 350 m a.s.l. in terms of texture, acidity, sweetness and aroma by means of a trained panel. Paprštein and co-workers[27] 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 appreciation in terms of several sensory attributes related to appearance, flavour and texture. None of them provided details about the sensory evaluation procedures. More recently, sensory analysis was applied in the horticultural sector as a reliable source of information to describe fruit quality, as affected by pre- and post-harvest factors.[28] Although sensory analysis is expensive, requiring time and resources, its fundamental role in the evaluation of food quality and consumer perception of food properties has been recognised. Many authors have underlined that analytical measurements are not always suitable for substituting sensory evaluation in screening food products and that human assessment should be the standard to calibrate instrumental readings,[29, 30] as humans can sometimes be more sensitive than machines[1, 31, 32] and give a more holistic response. Moreover, machines can measure one or several properties related to sensory perception, but only humans can provide "human responses".

Thus, in this work a detailed protocol combining quantitative descriptive analysis performed by a trained panel and tailored instrumental methods is proposed in order to measure apple texture with the objective of studying perceptible differences between apples grown at different altitudes and harvested at different times. To investigate changes in apple texture, our main focus in this study, 6 sensory attributes and 12 mechanical and 4 acoustic parameters were considered in a parallel manner. Cell anatomy analysis, which allows having information on tissue structure, was performed by microscopy in order to better interpret and understand the texture properties measured. In addition, perceived odour and flavour were also evaluated by the sensory panel to study how they evolve in different altitudes and their relationship with texture perception. The cross-effect of altitude and ripening on apple quality was examined by using fruit collected at three different harvest times.

## MATERIAL AND METHODS

### Plant material

Golden Delicious variety was chosen as having the larger production in Italy and a good adaption to different territory and climatic conditions. The apples were provided by the Laimburg Research Centre for Agriculture and Forestry, coming from three different orchards at 600 m a.s.l. (lower altitude, La, Lb, Lc) and three around 1000 m a.s.l. (higher altitude, Hd, He, Hf). The orchards are all located in the Val Venosta area (Alto Adige, Italy), within a range of 20 km, and the same growing practices were applied.

Heat sums from the six orchards were measured by calculating Growing Degree Days (GDDs) at a minimum threshold of 10 °C, starting from the data recorded by four meteorological stations: one for the La, Lb and Lc orchards, whereas there were three different stations for the Hd, He and Hf orchards.

For each orchard, three different harvest times were considered: T0, chosen as optimal for long-term controlled atmosphere storage by measuring basic parameters (firmness, soluble solid content, titratable acidity, starch index); T1 and T2, one and three weeks after T0 respectively. In total, 18 samples were tested (2 altitudes * 3 locations * 3 harvest dates). Information about the agronomical features and basic physico-chemical parameters is given in Tables 1 and 2 respectively. Fruit was stored for five months in refrigerated ultra low oxygen atmosphere conditions (1% $O_2$, 2.5% $CO_2$, 1.3°C and 98% of relative humidity). Then it was kept at room temperature for 24h before analysis.

**Sensory analysis**

Sensory analysis was performed based on an adaptation QDA® method[33]. The sensory lexicon was instead developed using the consensus method described by Murray et al[34]. They used a sensory vocabulary including six attributes for texture, two tastes, overall odour, overall retronasal flavour and astringency. Moreover, six specific attributes for odour and retronasal flavour were evaluated (Table 3). The intensity of each attribute was scored by the panel on a 100 mm linear scale, anchored at 0 (absence), 100 (extremely intense), and with 50 as middle point. Tests were carried out in a sensory laboratory equipped with 22 individual booths under artificial red light, by a trained panel composed of 17 panellists (6 males and 11 females), all employees at the Fondazione Edmund Mach (San Michele all'Adige, Trento, Italy). They were all part of the apple panel from several years (from 2 to 4 years). All panelists have been subjected to a 10-hour training before the analysis. Training consisted in several types of tests (recognition test, ranking test, comparative test, etc.) on all sensory categories in order to be sure that they were able to rate attributes using the linear scales anchored at the extremities with the product references. They were also trained to use the tasting protocol. Panel performances (discrimination, repeatability, agreement) were checked at the end of the training phase.

Samples were prepared according to the protocol reported in a previous paper[13]. Briefly, flesh cylinders (1.8 cm diameter; 1.2 cm height; ±2.5 g) were isolated from three apple slices cut around the equatorial plane perpendicular to the core. Each cylinder was immediately treated with an antioxidant solution (0.2% citric acid, 0.2% ascorbic acid, 0.5% calcium chloride). Fifteen apples per batch were cut to obtain a sufficient number of cylinders for 17 panelists. Each panellist received 8 apple cylinders per sample in a clear plastic cup encoded with a random three-digit code. Samples were presented monadically following a William's Latin square design. Sensory evaluations were performed within 1 h of sample preparation. Samples were analysed in three panel sessions. Panellists evaluated 6 samples out of 18 per session.

**Texture analysis**

Analysis was performed on ten flesh cylinders per sample (each cylinder from a different apple). The cylinders used for instrumental analyses came from the same fruits which provided cylinders for sensory analyses, in order to have data representative of what panellists perceived. Texture measurements consisted in compression test and were performed following the method by Costa et al.,[35] with 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 flesh cylinder, at a test speed of 300mm/min and compressing each cylinder at 90% of its height. Twelve mechanical and four acoustic parameters were calculated on the relative curves measured by the instrument (Table 4).

**Cell anatomy analysis**

Cell anatomy analysis is useful to study how fruit evolve and change in terms of structure during maturation at the different altitudes, in order to better interpret and understand the texture properties measured. Analysis of anatomical features was performed on fruit from T0. Cell volumes, cell numbers per fruit and the percentage of intercellular air spaces in each sample (n = 15 fruits) were assessed following the method by Goffinet et al.[36] Unlike other instrumental analyses, cell anatomy analysis was performed on different apples to the ones used for sensory evaluation, but coming from the same batch. Each fruit was cut along its equatorial line. Two wedge-shaped sectors were then sliced using a razor blade along the major and the minor radius of the cortex. Three pictures at 10× magnification were taken at 0.25, 0.50 and 0.75 of the length of each radius using a Leica DMLB light microscope equipped with a DC 300F camera, supported by IM1000 Image Manager software (Leica Microsystems AG, Heerbrugg, Switzerland). Pictures were analysed using ImageJ 1.45s software (National Institutes of Health, Bethesda, Maryland, USA), by applying a 11.000 pixel$^2$ grid, composed of nine rows and eleven columns. The cells and intercellular spaces within the grid were then counted. An example of photo used for this analysis can be seen in Fig 1.

**Basic chemical composition**

Soluble solid content (SSC) and titratable acidity (TA) were measured in duplicate on the juice squeezed from eight cylinders sampled from different fruit (the same used simultaneously for sensory measurement). For SSC, expressed in ° Brix, a DBR35 refractometer (XS Instruments, Poncarale, Brescia, Italy) was used. TA, expressed as malic acid equivalents per 100 g of juice, was measured using a Compact Titrator (Crison Instruments S.A., Alella, Barcelona, Spain) with 0.1N sodium hydroxide solution (to pH 8.16).

**Statistical analysis**

Two-factor ANOVA with interaction was performed on sensory and instrumental data, considering altitude and time of harvest as experimental factors, and one-way ANOVA on cell counting data, using STATISTICA 9.1 software (StatSoft, Inc., USA). *P*-values equal to or lower than 0.05 were considered significant. When significant differences were observed, mean comparison tests were performed consecutively using Tukey's HSD test with a confidence level of 95%. Generalized Procrustes Analysis (GPA) was performed on sensory data with Senstools 3.1.6 software (OP&P Product Research BV, Utrecht, the Netherlands). The biplot was used to examine the relations between the sensory variables and the products and relative positioning of the products to each other's.

## RESULTS

**Texture properties**

Two-factor ANOVA on sensory data indicates significant differences for altitude and harvest time factors. The texture attributes crunchiness, flouriness, fibrousness and graininess present differences for both factors (Table 5). In contrast, hardness and juiciness are only significant for one of the two factors, time of harvest and altitude respectively. Considering the harvest-time factor, T2 samples present a lower intensity for hardness, crunchiness, fibrousness and a higher intensity for flouriness and graininess compared to earlier harvest-times, T0 and T1. As regards the altitude factor, samples from lower altitude were perceived to be juicier, crunchier and more fibrous than samples from higher altitude, which were more floury and grainy. Even though not significant, there is a tendency towards decreasing juiciness with increasing time ($p = 0.088$) and decreasing hardness with increasing altitude ($p = 0.069$). No significant interaction is found between time of harvest and altitude demonstrating that the differences observed in the samples in relation to the two factors are changing in the same direction (Table 5).

Two-way ANOVA on instrumental data from the texture analyser is in agreement with the sensory description. Significant differences can be observed for texture parameters, both mechanical and acoustic, when looking at the time of harvest factor (Table 6). In particular, a gradual decrease is seen as the time of harvest increases from T0 to T2. A significant altitude effect is seen for the majority of textural (mechanical and acoustic) parameters, with samples from lower altitude having a higher response than samples from higher altitude. Four mechanical parameters also indicate 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 6).

One-way ANOVA on cell anatomical data shows that fruit from lower altitude has a higher cell number and a higher percentage of intercellular spaces (Table 7). Even when fruit weight is not significantly different, the volume of fruit from low altitude is higher than fruit from high altitude (Table 7).

**Impact on flavour perception**

In Table 5, it can be observed that only one sensory attribute, honey odour, presents a significant effect of the altitude factor. This latter factor induces then a little impact on flavour perception. On the contrary, the time of harvest factor has a stronger effect. Indeed, significant differences between products can be seen for six attributes (odour: 2, taste: 2, and flavour: 2).

ANOVA results on SSC and TA data partly corroborate the sensory description (Table 6). TA varies significantly depending on the time of harvest. In particular, samples from T2 are found to have a lower acid concentration than T0 and T1. A significant altitude effect is observed for SSC, with samples from lower altitude having higher SSC (Table 6).

**Overall sensory view: greater effect of time of harvest**

The relative sensory differences between all products are graphically represented in the biplot (scores and loadings) from GPA, performed on the general profile and the odour/flavour sensory datasets separately (Figures 2A and 2B respectively) for better interpretation. In Figure 2A, 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 appears to be more related to sweet and sour taste, with samples having higher sweetness in the lower part of the plot. In general, samples from higher altitude appear to be located in the upper side of the plot, excepted for Hf samples. In contrast, samples from lower altitude appear to be located in the lower part. Thus, samples from lower altitude are generally described as sweeter and juicier than samples from higher altitude, which appear to be sourer and more astringent. The odour and flavour profile depicted in Figure 2B does not reveal sample distribution related to altitude, but 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 are described as having mainly grass and lemon odour/flavour. Significant differences are perceived for pear and banana odours and for banana flavour, which are found to have a higher intensity in T2 than T0. Lemon flavour is higher in T0 than T2.

**Heat sums contribute to climatic conditions**

Temperature is a factor of great importance in climatic conditions. In our study, we took this factor into account through the GDD index. The average temperatures at 1000 m a.s.l. were generally lower than those at 600 m a.s.l. throughout the growing season, as illustrated by the heat sums in Figure 3A. As presented in Figure 3B, GDDs at the beginning of the growing season were higher for fruit at low altitude. The GDDs measured for Hf, in particular, tended to be closer to those measured for low altitude samples.

## DISCUSSION

Our results show that the time of harvest impacts sensory characteristics and global fruit quality more than altitude. Nevertheless, this second factor significantly affects perceived texture properties, which are crucial to consider in order to obtain apples corresponding to consumer expectations.

Ferrandino et al. analysed 'Golden Delicious' apples from three different altitudes (350, 750, 1000 m a.s.l.) and found that a different volatile compound (VOC) profile was produced by the fruit in the different climatic environments.[25] They found higher development of volatiles in fruit from 1000 m a.s.l. These aspects are also important to consider when aiming at improving apple quality. However, this topic was not the main subject of this paper as it mainly put attention to texture properties and would need further investigations. But, it should be noted that our data are not drawing the same conclusions when looking at flavour sensory results. Altitude did not seem to affect VOC perception as only honey odour showed significant differences, with samples from high altitude having a higher intensity than those from low altitude (Table 5).

In our study, it was observed that fruit from lower altitude had a higher number of cells, intercellular spaces and a larger total volume. The authors hypothesise that cell division lasts longer in fruit from low altitude, with a higher number of cell replications compared to fruit from higher altitude. This could cause an increase in fruit expansion at low altitude. Warrington et al. demonstrated that different ranges 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.[37] The fruit was found to be bigger when temperatures were higher. They also showed higher SSC, and a decrease in flesh firmness was found as the temperatures increased. Stanley et al. 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.[38] This is explained by the fruit growth mechanism, which involves an early exponential cell division phase, lasting for the first week. A phase of contemporary cell division and cell expansion then follows, lasting for about 3-4 weeks, and finally a phase of cell expansion only characterises the rest of the season.[39] Our temperature measurements (by means of GDDs) corroborate these results. At the beginning of the growing season GDDs were higher at lower altitude. The GDDs for Hf samples, in particular, tended to be closer to those measured for lower altitude samples. Thus, the temperature effect could explain the sensory description of Hf samples, which were generally evaluated as more similar to lower altitude fruit, as compared to the other two higher altitude samples (Fig. 2A).

Besides, it has been demonstrated in the literature that fruit size can be modulated by several factors. Fruit size is associated negatively with crop load[40, 41] and positively with maturity levels at harvest. [42]

In our study, no relationship was found between cell volume and fruit size (data not shown), corroborating results coming from other studies.[36, 43] Harada et al., in particular, demonstrated that final fruit volume in *Malus Domestica* is mainly related to cell proliferation rather than cell enlargement.[43] This is a further confirmation of our study results, showing higher number of cells per fruit in larger fruits (Table 7).

The anatomical data, carried out here by means of cell analysis, were consistent with the apple consistency sensory description and instrumental measurements performed with the texture analyser. The higher the number of cells, the higher is the force required to compress the sample. Moreover, a higher number of cells means a higher level of cell wall crushing during compression. Cell wall rupture and the expansion of liquid content under pressure is responsible for the sound emitted by wet foods when they are crushed.[44] Furthermore, fruit from lower altitude presented a 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 greater the sound, because the expansion of cell liquid in the surrounding empty spaces causes noise emission. The sound produced when food is crushed is strongly related to the sensory perception of crunchiness.[45] Our sensory data are in agreement with these observations, since crunchiness intensity was higher in samples from lower altitude.

Concerning juiciness, we observed a decrease when altitude and ripening are increasing. Juiciness does not seem to be linked to the number or size of cells but more to how the cell walls are deforming themselves during mastication and mechanical testing.[46, 47] In firm apples, tissue fracture is associated with breakage of individual cells and results in the release of cell fluids. However, in soft and mealy apples, fractures occur as a result of cell-to-cell debonding and thus individual cells do not always break open or release their contents ("juice").[47, 48] Juiciness remains a characteristic difficult to predict probably because of the underlying phenomena which are still not fully understood.[1, 49]

It is important to consider that this study was performed in a single apple season. This is an important limitation in terms of providing effective conclusions about the real quality of fruit grown in different climatic conditions.

## CONCLUSIONS

Important differences in texture - more specifically in crunchiness, flouriness, fibrousness and graininess - among samples from the six orchards were found not only as a consequence of time of harvest, but also related to altitude. Apples from higher altitude showed a lower fruit volume, with a lower cell number and percentage of intercellular spaces, probably due to different early season temperatures causing different cell division patterns. This was responsible for different texture properties, and these differences were perceivable by human senses.

These preliminary results suggest that the differences in terms of anatomical and structural features developed by apples grown in different climatic conditions, including different altitudes, can be perceived by human senses and that the sensory-instrumental tool applied here provided useful information for describing such differences. In addition to texture measurements, the cell counting method provided complementary data helping to explain sensory perception. Thus, proper sensory evaluation is advised  when studying the effect of agronomical factors, in order to obtain a reliable description of organoleptic properties perception of the final product. By providing a better understanding about the influence of altitude and time of harvest on apple sensory properties, this work could support the apple producers, from semi-mountainous regions (e.g. Alpes, Tyrol, etc.), in advertising and valorising their products with their specific characteristics in a more efficient manner.


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**Table 1:** Agronomical data from the six orchards under study.

| Code | m a.s.l. | Year planting | Light exposure | % slope | Crop load (t/ha) | Full bloom | Date T0 | Date T1 | Date T2 |
|---|---|---|---|---|---|---|---|---|---|
| La | 652 | 2010 | N | 8.5 | 85.3 | 01/04/2012 | 19/09/2012 | 25/09/2012 | 10/10/2012 |
| Lb | 656 | 2009 | N | 11.0 | 95.4 | 01/04/2012 | 19/09/2012 | 25/09/2012 | 10/10/2012 |
| Lc | 580 | 2003 | N | 11.4 | 98.2 | 01/04/2012 | 19/09/2012 | 25/09/2012 | 10/10/2012 |
| Hd | 1070 | 2002 | S | 9.3 | 91.4 | 18/04/2012 | 26/09/2012 | 02/10/2012 | 16/10/2012 |
| He | 1040 | 2010 | S | 13.2 | 52.4 | 13/04/2012 | 26/09/2012 | 02/10/2012 | 16/10/2012 |
| Hf | 1070 | 2010 | S | 15.8 | 69.6 | 13/04/2012 | 26/09/2012 | 02/10/2012 | 16/10/2012 |

T0, T1 and T2 refer to the harvest dates: T0=optimal harvest for long-term controlled atmosphere storage, T1=T0+1 week, T2=T0+3 weeks. Samples from orchards around 600 m a.s.l. are considered from low altitude (L); samples from orchards around 1000 m a.s.l. are considered from high altitude (H); the 6 orchards are coded by letter from a to f.

**Table 2:** Instrumental characterisations: mean values and standard deviation for basic physico-chemical data for the 3 harvest dates.

| Code | Harvest time | Firmness ($kg/cm^2$) | Soluble Solid Content (°Brix) | Titratable Acidity (g/L) | Starch Index (1-5) |
|---|---|---|---|---|---|
| La | T0 | 6.29 ± 0.51 | 12.47 ± 0.92 | 5.43 ± 0.17 | NA |
| Lb | T0 | 6.10 ± 0.47 | 12.63 ±0.62 | 5.07 ± 0.12 | NA |
| Lc | T0 | 6.41 ± 0.49 | 13.03 ± 0.90 | 6.00 ± 0.14 | NA |
| Ha | T0 | 7.02 ± 0.70 | 12.13 ± 0.59 | 5.83 ± 0.17 | 2.7 ± 0.35 |
| Hb | T0 | 6.44 ± 1.11 | 13.07 ± 0.67 | 6.40 ± 0.16 | 2.9 ± 0.53 |
| Hc | T0 | 6.57 ± 0.48 | 14.07 ± 0.49 | 6.27 ± 0.12 | 2.8 ± 0.16 |
| La | T1 | 6.16 ± 0.68 | 14.10 ± 0.82 | 5.30 ± 0.22 | 3.1 ± 0.68 |
| Lb | T1 | 6.06 ± 0.55 | 12.87 ± 0.77 | 4.10 ± 0.16 | 3.9 ± 0.91 |
| Lc | T1 | 6.32 ± 0.50 | 12.60 ± 1.22 | 4.33 ± 0.09 | 4.0 ± 0.81 |
| Ha | T1 | 6.44 ± 0.54 | 12.30 ± 0.74 | 5.33 ± 0.19 | 3.8 ± 0.04 |
| Hb | T1 | 6.29 ± 0.74 | 13.40 ± 0.60 | 5.73 ± 0.21 | 3.8 ± 0.11 |
| Hc | T1 | 6.41 ± 0.61 | 14.03 ± 0.53 | 5.83 ± 0.12 | 3.6 ± 0.00 |
| La | T2 | 5.58 ± 0.94 | 14.03 ± 1.21 | 4.30 ± 0.14 | 4.1 ± 0.12 |
| Lb | T2 | 5.51 ± 0.49 | 13.63 ± 0.95 | 3.77 ± 0.12 | 4.2 ± 0.08 |
| Lc | T2 | 5.53 ± 0.76 | 13.27 ± 0.82 | 3.63 ± 0.05 | 4.7 ± 0.08 |
| Ha | T2 | 6.33 ± 0.47 | 13.13 ± 0.68 | 5.37 ± 0.12 | 4.3 ± 0.46 |
| Hb | T2 | 5.42 ± 0.87 | 13.07 ± 0.47 | 4.97 ± 0.05 | 4.8 ± 0.16 |
| Hc | T2 | 6.08 ± 0.58 | 14.73 ± 0.73 | 5.33 ± 0.17 | 4.6 ± 0.30 |

T0, T1 and T2 refer to the harvest dates: T0=optimal harvest for long-term controlled atmosphere storage, T1=T0+1 week, T2=T0+3 weeks.
Samples from orchards around 600 m a.s.l. are considered from low altitude (L); samples from orchards around 1000 m a.s.l. are considered from high altitude (H); the 6 orchards are coded by letter from a to f.

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

| **Category** | **Attributes (english) /** ***Italian translation*** | **Definition** |
|---|---|---|
| Texture | Hardness / *Durezza* | Resistance of the sample at the first chew with molars |
| Texture | Juiciness / *Succosità* | Amount of juice released during chewing (first three chews) |
| Texture | Crunchiness / *Croccantezza* | Sound (pitch/intensity) produced by the sample during 5 molar chews |
| Texture | Flouriness / *Farinosità* | Degree of flesh breaking in small and dry fragments/granules during chewing |
| Texture | Fibrousness / *Fibrosità* | Degree of flesh breaking during chewing in thick and fibrous fragments/granules |
| Texture | Graininess / *Granulosità* | Numbers/size of fragments/granules produced during chewing |
| Flavour | Sweet taste / *Dolcezza* | Sweet taste sensation |
| Flavour | Sour taste / *Acidità* | Sour taste sensation |
| Flavour | Astringency / *Astringenza* | Tactile dryness sensation in the mouth (at the end of mastication) |
| Flavour | Overall Odour / *Odore Complessivo* | Overall odour sensation perceived via the orthonasal route |
| Flavour | Overall Flavour / *Flavour Complessivo* | Overall flavour sensation perceived via the retronasal route |
| Flavour | Pear /*Pera* | Specific odour (Od) or retronasal flavour (Fl) sensation[a] |
| Flavour | Banana / *Banana* | Specific odour (Od) or retronasal flavour (Fl) sensation |
| Flavour | Lemon / *Limone* | Specific odour (Od) or retronasal flavour (Fl) sensation |
| Flavour | Grass / *Erba* | Specific odour (Od) or retronasal flavour (Fl) sensation |
| Flavour | Vanilla / *Vaniglia* | Specific odour (Od) or retronasal flavour (Fl) sensation |
| Flavour | Honey / *Miele* | Specific odour (Od) or retronasal flavour (Fl) sensation |

* ‘Od’ and ‘Fl’ refer to codings in Fig. 3b, to differentiate between odour and retronasal flavour attributes.

**Table 4:** Mechanical and acoustic parameters extracted from the texture analyser curves while performing compression measurements, following the method by Costa et al. (2011).

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

**Table 5:** Mean values and *p*-values from two-way ANOVA on sensory data, considering harvest time and altitude as factors.

| | | Time of harvest | | | | Altitude | | | Time of harvest*Altitude |
|---|---|---|---|---|---|---|---|---|---|
| **Category** | **Attribute** | **T0** | **T1** | **T2** | ***p*-value** | **L** | **H** | ***p*-value** | ***p*-value** |
| Texture | 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 |
| Flavour | 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 | | 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 |
| | 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 |

In bold are reported ANOVA *p*-value ≤ 0.05. HSD tests were used with a confidence level of 95%; different letters indicate significant differences.

**Table 6:** Mean values and *p*-values from two-way ANOVA on instrumental data, considering harvest time and altitude as factors.

| | | Time of harvest | | | | Altitude | | | Time of harvest*Altitude |
|---|---|---|---|---|---|---|---|---|---|
| **Measurements** | **Parameter** | **T0** | **T1** | **T2** | ***p*-value** | **L** | **H** | ***p*-value** | ***p*-value** |
| Texture: mechanical and acoustic | 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 |
| Chemical composition | SSC | 15.4 | 14.5 | 15.0 | 0.167 | 16.2 b | 13.7 a | **0.000** | 0.092 |
| | TA | 8.0 a | 7.3 a | 5.9 b | **0.000** | 7.3 | 6.9 | 0.323 | 0.637 |

In bold are reported ANOVA *p*-value ≤ 0.05. HSD tests were used with a confidence level of 95%; different letters indicate significant differences. For texture analyser parameters coding, see Table 4.

**Table 7:** Mean estimated values and *p*-values from ANOVA for number of cells per fruit, percentage of air spaces and fruit volume from anatomical measures on fruit from lower and higher altitude.

| | Altitude | | |
|---|---|---|---|
| **Parameter** | **Low** | **High** | ***p*-value** |
| Nr. Cells (millions) | 95.3 b | 82.7 a | **0,000** |
| % Air Spaces | 15.96 b | 13.76 a | **0,002** |
| Fruit volume ($mm^3$) | 273 b | 246 a | **0,000** |

In bold are reported p-value ≤ 0.05. HSD tests were used with a confidence level of 95%; different letters indicate significant differences.

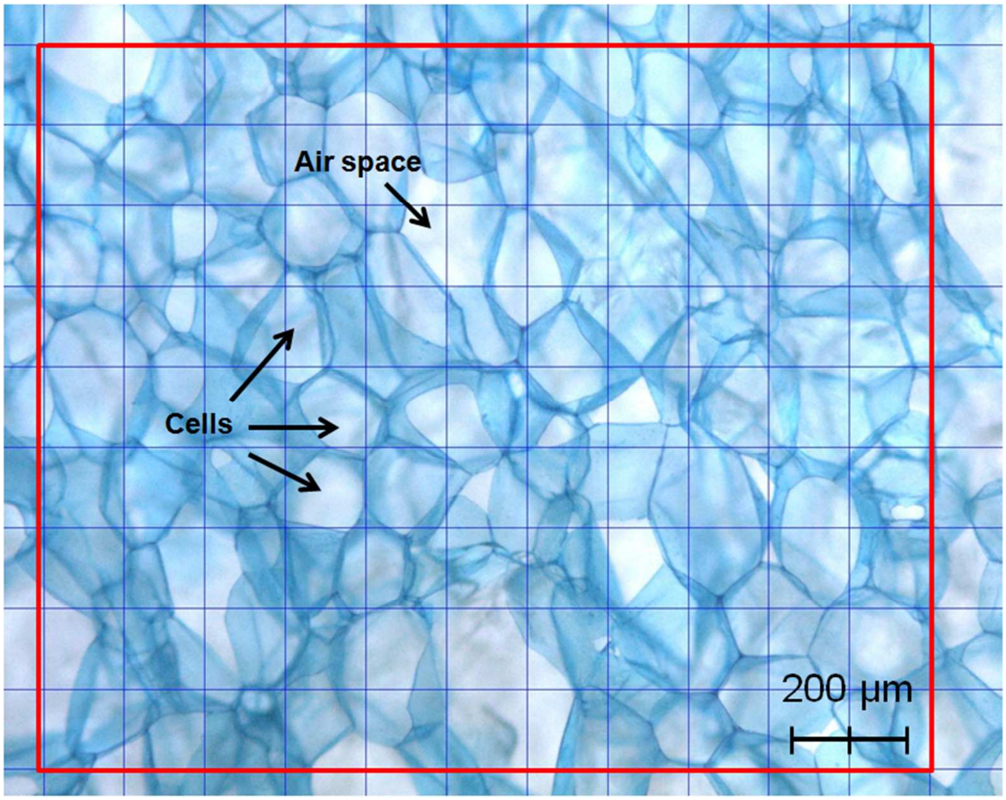


Fig. 1: Example of apple tissue photo used for cell anatomy analysis.

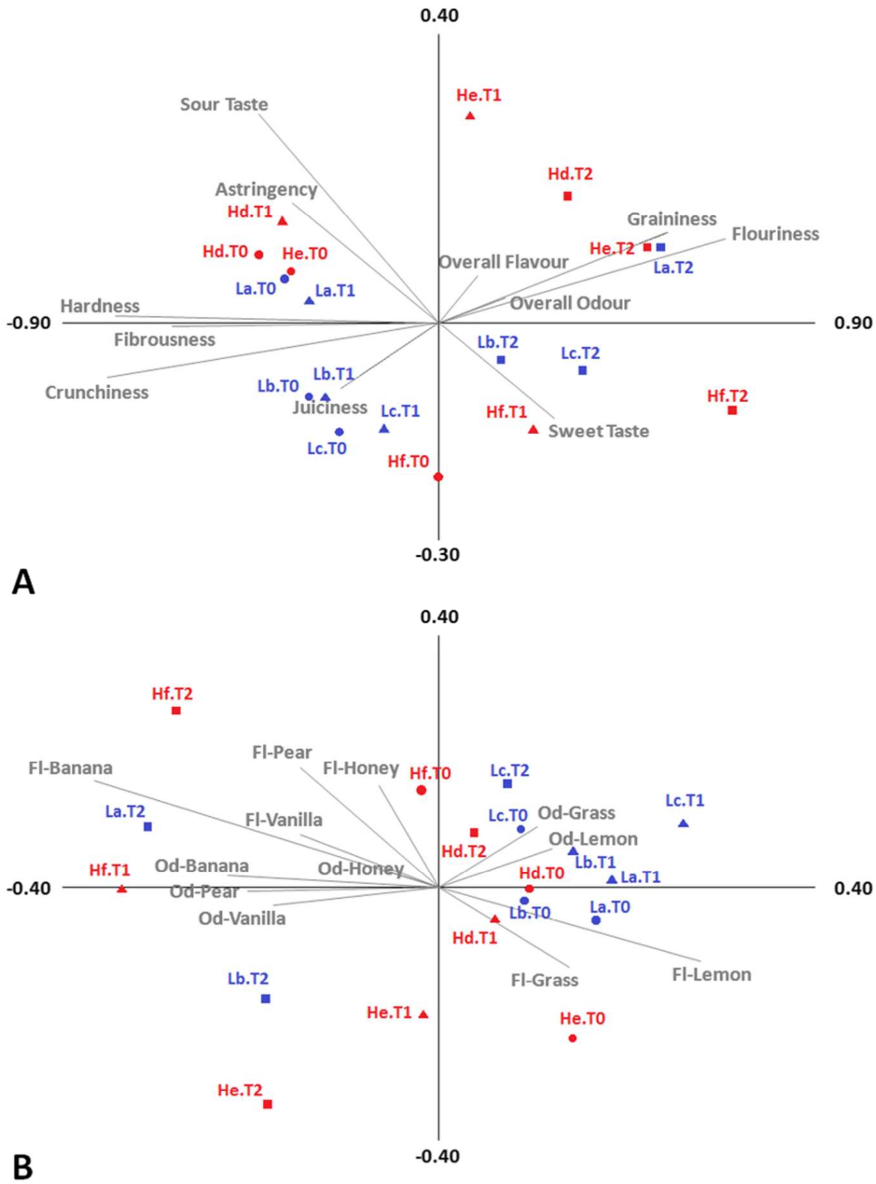


Fig. 2: GPA bi-plots (Dim.1 vs. Dim.2) of basic profile (A; Dim.1: 49%; Dim.2: 10%) and specific odour (Od) and retronasal flavour (Fl) profile (B; Dim.1: 21%; Dim.2: 15%). Samples from lower altitude are indicated by blue markers; samples from higher altitude by red markers. Circle markers are for T0; triangles are for T1; squares are for T2.

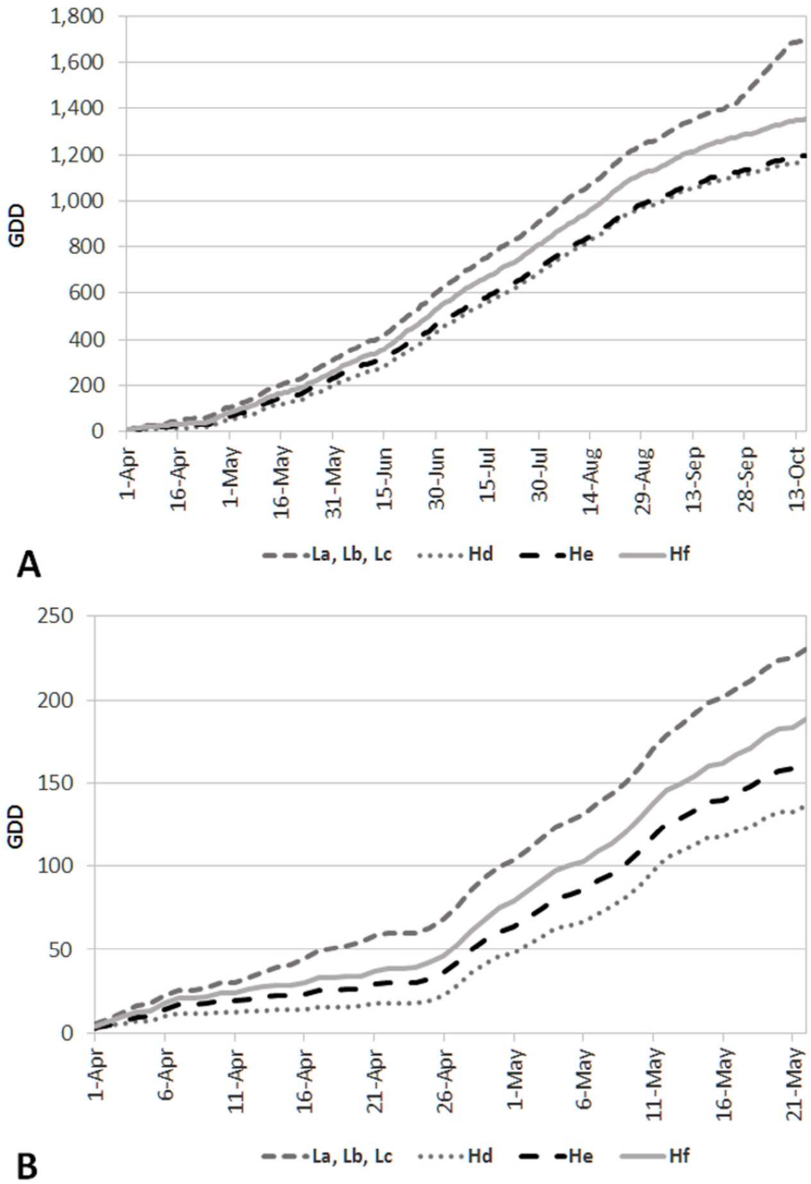


Fig. 3: Heat sums calculated for the six orchards at lower and higher altitudes. Data are from the entire fruit growing period, until harvest, in panel A; from the cell division phase period in panel B. One meteorological station only recorded data for La, Lb, and Lc; three different stations recorded data for Hd, He, and Hf orchards.