

A combined sensory-instrumental tool for apple quality evaluation

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14 **Abstract**

15

16 A combined approach for perceptible quality profiling of apples based on sensory and instrumental
17 techniques was developed. This work studied the correlation between sensory and instrumental
18 data, and defined proper models for predicting sensory properties through instrumental
19 measurements. Descriptive sensory analysis performed by a trained panel was carried out during
20 two consecutive years, on a total of 27 apple cultivars assessed after two months postharvest
21 storage. The 11 attributes included in the sensory vocabulary discriminated among the different
22 apple cultivars by describing their sensory properties. Simultaneous instrumental profiling including
23 colorimeter, texture analyser (measuring mechanical and acoustic parameters) and basic chemical
24 measurements, provided a description of the cultivars consistent with the sensory profiles.
25 Regression analyses showed effective predictive models for all sensory attributes ($Q^2 \geq 0.8$), except
26 for green flesh colour and astringency, that were less effective ($Q^2 = 0.5$ for both). Interesting
27 relationships were found between taste perception and flesh appearance, and the combination of
28 chemical and colorimeter data led to the development of an effective prediction model for sweet
29 taste. Thus, the innovative sensory-instrumental tool described here can be proposed for the reliable
30 prediction of apple sensory properties.

31

32 **Keywords:** Apple (*Malus × domestica* Borkh.); Sensory profiling; Physical properties; Chemical
33 properties; Prediction models

34

35 **1 Introduction**

36

37 Texture properties of fruit and vegetables are considered the most important drivers of consumer
38 choice, followed by flavour characteristics (Dailliant-Spinnler et al., 1996; Jaeger et al., 1998;
39 Péneau et al., 2006 and 2007; Harker et al., 2008). Food suppliers currently measure apple quality

40 by considering basic pomological descriptors, such as fruit shape, size, colour, soluble solids
41 content, titratable acidity, and by penetrometer-assessed fruit firmness, the most frequently used
42 method for measuring fruit mechanical properties (Harker et al., 1997; Hoehn et al., 2003). Sensory
43 analysis is not usually considered: it is expensive and limited to a small number of samples because
44 it employs humans as sensory instruments. Moreover, it cannot be used for measuring quality
45 properties in real time, an aspect particularly important for agricultural products, since their high
46 variability require large sampling schemes. Moreover, the quality assessment of breeding material,
47 normally represented by a single plant/individual, can be restricted by sample availability, which is
48 often not sufficient for sensory panel evaluations. However, the best way to precisely describe the
49 eating quality of food is still the sensory approach, which is able to define, measure, quantify, and
50 explain what is really perceivable by human senses (Carbonell et al., 2008). Sensory analysis, in
51 fact, provides a comprehensive description of a product (Murray et al., 2001). To overcome these
52 limitations, and to allow quality characterisation on a large sample set, the prediction of sensory
53 attributes by instrumental measures would represent a much needed innovation in quality control.
54 The majority of recent studies address texture properties (De Belie et al., 2002; Harker et al., 2002a;
55 Mehinagic et al., 2003; Chauvin et al., 2010). Harker et al. (2002a) through various instrumental
56 measures to predict texture sensory attributes, show the possibility to predict sensory firmness,
57 crispness, crunchiness, initial juiciness, and ease of breakdown through a puncture test. They also
58 showed that a difference of 6-8 N in instrumental firmness is necessary before it can be perceived
59 by a trained sensory panel (Harker et al., 2002a). Chauvin et al. (2010) found a strong correlation
60 between texture sensory attributes and compression measurement by texture analyser. Mehinagic et
61 al. (2003) compared the use of a penetrometer with non-destructive vis/NIR analyses, focusing on
62 the correlations with sensory assessments, in order to propose a non-destructive measurement as a
63 valid alternative. Brookfield et al. (2011) proposed the use of small panels (< 4 subjects) as a
64 cheaper alternative to measure apple texture, finding that a panel is efficient only if it concentrates on
65 a very small number of attributes (such as crispness and juiciness). The same authors also

66 highlighted that the instrumental-sensory relationship did not follow a unique trend, because each
67 cultivar tends to respond differently to different tests (Brookfield et al., 2011). This observation
68 suggests that a large set of apple cultivars, representing a wide range of variability for several
69 sensory apple attributes, should be considered in such studies. Human assessment should always be
70 considered as a reference to calibrate instrument readings, in order to develop tools falling within
71 the range of textural parameters known to be accepted by consumers (Bourne, 2002; Harker et al.,
72 2003).

73 While perceived texture can sometimes be predicted by instrumental data, flavour and taste
74 attributes are, generally, more difficult. For instance, many studies, underline the difficulties in
75 developing a reliable model to predict sweet taste, finding conflicting results between sweetness and
76 texture properties (Harker et al., 2002b; Harker et al., 2006; Echeverría et al., 2008). Any sensory
77 attribute could indeed have a potential influence on the perception of other properties not directly
78 related to it. This is particularly true in the case of flavours, which derive from the integration of
79 different senses (taste, smell and tactile stimuli; see Prescott, 2012; Small, 2012).

80 Recently, Costa et al. (2011) proposed the use of a texture analyser to dissect apple fruit texture into
81 several components by simultaneously profiling mechanical and acoustic components. The method
82 was tested on 86 different apple cultivars, and the data were compared with the sensory texture
83 profiles provided by a restricted panel of experts, evaluating 21 apple cultivars for firmness,
84 crispness, and juiciness attributes. Regression analyses highlighted that the instrumental force
85 parameters from texture analyser measurements were necessary to predict both firmness and
86 crispness sensory attributes, and that a high correlation between acoustic parameters and the sensory
87 attribute of crispness does effectively exist (Costa et al., 2011).

88 In this study we propose a complete methodology for sensory profiling of apples. This was applied
89 in parallel to instrumental measurements of specific physical and chemical properties, including
90 texture analyser measurements (as proposed by Costa et al., 2011), dry matter concentration,
91 extractable juice content, colorimeter measurements, and basic chemical composition. This

investigation was carried out for two consecutive years on a wide selection of apple cultivars, in order to study the sensory profiles of apples having the highest possible variability in their sensory properties. Based on the correlation between sensory and instrumental data, our scope was to propose a new effective approach for the prediction of sensory properties through instrumental characterisation.

2 Materials and methods

2.1 Plant Materials

A set of 27 commercial apple cultivars (*Malus × domestica* Borkh.), commonly grown and commercialized in Italy, was analysed over two years (2010 and 2011), with 18 common cultivars shared between the two experimental years. Six cultivars in 2010 and two in 2011 were evaluated twice, since they were harvested from different orchards (Table 1). In 2011, two additional clones were analysed for two cultivars: Roho 3615 for Pinova cultivar and Red Spur Jeromine for Red Delicious. All orchards were managed according to standard agronomical practices for thinning, pruning, disease and pest control. Fruit were picked at commercial harvest, determined by the standard descriptors used to monitor fruit maturity and ripening, such as flesh firmness, skin colour and starch degradation index. The instrumental parameters were monitored on fruit samplings starting from 10 days before the supposed optimum date (Asrey et al., 2008). References for each cultivar were provided by Consorzio delle Cooperative Ortofrutticole dell'Alto Adige (Werth, 1995). 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.

2.1.1 Sample preparation

118 Samples were prepared according to the protocol reported in Corollaro et al. (2013). Briefly, flesh
119 cylinders (1.8 cm diameter; 1.2 cm height) were isolated from three apple slices cut around the
120 equatorial plane perpendicular to the core. Each cylinder was immediately treated with an
121 antioxidant solution (0.2% citric acid, 0.2% ascorbic acid, 0.5% calcium chloride). Cylinders
122 coming from the same fruit were used for both sensory (8 cylinders put into clear plastic cups
123 encoded with a random three-digit code) and instrumental analyses. Sensory evaluations were
124 performed within one hour of sample preparation, while instrumental analyses were carried out
125 within three hours, keeping the samples in sealed containers in refrigerated condition until
126 measurement. Apart from fruit weight, measured the day before the sensory analysis, all other
127 sensory and instrumental measurements were performed after the antioxidant treatment in order to
128 compare instrumental and sensory data.

129

130 *2.1.2 Preliminary validation of sample preparation procedure*

131 In order to study any possible influence of the antioxidant solution on sample sensory properties,
132 discriminate analysis was performed by a trained panel (15 males, 15 females; all FEM employees)
133 according to the standard triangle test procedure (ISO, 2004). Three different apple cultivars known
134 to be very different in terms of sweetness/acidity were chosen: ‘Fuji’ (high sweetness – low
135 acidity), ‘Cripps Pink’ (medium sweetness – medium acidity), ‘Granny Smith’ (high acidity – low
136 sweetness).

137 For each of the 3 cultivars, the triangle test compared samples treated with the antioxidant solution
138 and samples treated with water to prevent the judges from perceiving visual differences related to
139 surface moisture. The three paired samples were presented to the judges following a balanced
140 design. In addition, the test was performed under red light to mask any possible browning defects
141 in the samples not treated with the antioxidant solution. Test implementation, recording judges’
142 responses and data analysis were performed with FIZZ software 2.46A (Biosystemes, France).

143 Titratable acidity and soluble solids content were measured in triplicate on the juice expressed by
144 mechanical compression from flesh cylinders from treated and untreated apples (6 fruit per sample)
145 following the procedures described in 2.3.4.

146

147 **2.2 Sensory analysis**

148

149 The trained sensory panel included 13 judges in 2010 (6 males; 7 females) and 14 in 2011 (4 males;
150 10 females), all FEM employees, with seven judges in common for both years. Sensory profiling
151 was performed based on the quantitative descriptive method reported by Stone and Sidel (2004).
152 The sensory lexicon was instead developed using the consensus method of Murray et al. (2001). In
153 2010, the sensory vocabulary was composed of attributes related to flesh colour, odour, texture, and
154 flavour. Details about panel training, and about definition, evaluation procedure, and reference
155 standards for each attribute are reported in Corollaro et al. (2013). Odours (orthonasal perceptions
156 by smelling) and flavours-by-mouth (retronasal perceptions by tasting) were evaluated both by the
157 overall intensity and by a set of 31 specific attributes (Aprea et al., 2012). Lexicon was the same in
158 2011 as in 2010, with the exception of “bitter taste”, which was removed as it was not discriminant,
159 and “crispness”, which was redundant due to its strong positive correlation with crunchiness ($r =$
160 0.99 ; $p < 0.001$). Therefore, the specific sensory attributes for odour and flavour-by-mouth were
161 reduced to nine.

162 In this study, only the 11 attributes related to appearance (2), texture (6), and flavour (3) common to
163 both seasons were considered (Table 4), while the profiles related to specific odour and flavour-by-
164 mouth attributes were preliminary investigated in Aprea et al. (2012).

165 The intensity of each attribute was scored by the panel on a 100 mm linear scale, anchored at 0
166 (absence), 100 (extremely intense), and with 50 as middle point. The sensory tests were performed
167 once per week (in a few cases, twice a week) from October to December in 2010 and 2011 (dates in
168 Table 1) in individual computerised booths equipped with FIZZ software (2.46A, Biosystemes,

169 Couternon, France) under white artificial lighting. Unsalted bread and water were provided to the
170 judges to cleanse their palate between samples. Six apple samples (three cultivars replicated twice)
171 were analysed per session, according to a randomised balanced order of the judges
172

173 **2.3 Instrumental analyses**

175 *2.3.1 Colour analysis*

176 L*a*b components from CIELAB colour space model (see Schanda, 2007) were measured on four
177 samples of flesh cut from each fruit using a CR-400 colorimeter, supported by the CM-S100w
178 SpectraMagic™ colour data software (Konica Minolta Sensing, Inc., Japan).
179

180 *2.3.2 Texture analysis*

181 Texture properties were measured on flesh cylinders (ten cylinders sampled from ten different fruit
182 per each cultivar; each cylinder was considered a replicate of that cultivar) by a TA-XT texture
183 analyser equipped with an acoustic envelop detector device (Stable MicroSystem Ltd., Godalming,
184 UK). A 4 mm probe was used to compress the samples. Twelve mechanical and four acoustic
185 parameters were calculated on the recorded curves, following the method described by Costa et al.
186 (2011; Table 5; supplementary data Table S1).
187

188 *2.3.3 Juice extraction and dry matter concentration*

189 Extractable juice was measured in duplicate by weighing the liquid expressed from mechanical
190 compression of eight flesh cylinders per cultivar (each cylinder coming from a different fruit). Dry
191 matter concentration was measured by drying a sample of eight flesh cylinders per cultivar at 105°C
192 until they reached stable weight. Both were expressed as percentage of fresh weight (Supplementary
193 data Table S1).
194

195 2.3.4 Basic chemical measurements

196 The concentration of soluble solids (%SSC) and titratable acidity was measured on the juice
197 expressed from mechanical compression of 12 cylinders sampled each from different fruit. The
198 measures were performed in two replicates with a DBR35 refractometer (XS Instruments,
199 Poncarale, Brescia, Italy) and with a Compact Titrator (Crison Instruments S.A., Alella, Barcelona,
200 Spain), respectively (Supplementary data Table S1). NaOH 0.1N was used to titrate 5g of juice to
201 pH 8.16. The results were calculated as malic acid equivalents in 100g juice.

203 2.4 Statistical analysis

205 Panel performances were evaluated on both 2010 and 2011 data-sets to validate the sensory method
206 through a three-way mixed ANOVA applied to the individual scores (considering judge as a
207 random factor, and product and replicate as fixed factors).

208 Statistical analysis of triangle data was based on binomial distribution with a guessing probability p
209 $= 1*3^{-1}$.

210 For the following analysis the scores for each attributes were averaged over panel. The sensory and
211 instrumental profiles of the complete data-set including both years (except for specific analyses
212 applied on 2011 data only, as indicated below) were evaluated using univariate and multivariate
213 approaches.

214 First of all, sensory data were explored by Principal Component Analysis (PCA), performed on a
215 data correlation matrix. Data were mean centered and scaled to unit variance. Pearson's correlations
216 among both sensory attributes and instrumental parameters (n corresponding to the number of
217 samples available for each variable) were performed for exploring variable bi-variate linear
218 relations. Differences among apple samples in terms of instrumental parameters were estimated by
219 means of one-way ANOVA model (with the exception of dry matter concentration, where no
220 replicates were available), considering product as fixed factor (p -value lower than 0.05 indicated

significant differences; no Post-Hoc tests were used). Partial Least Square Analysis 2 (PLS-2) was used to explore the relationship among all sensory and instrumental variables. It is an estimated regression model maximising the covariance between two data matrices: Y (matrix of dependent variables) to X (matrix of independent variables). In order to study the relationship between a single sensory attribute and instrumental parameters, the method of partial Least Square Analysis 1 (PLS-1) was used instead. It is a regression model where one single y-dependent variable is related to two or more x independent variables. The model is estimated maximising the covariance between the X matrix and the single y-column. Thus, PLS-2 and PLS-1 were both applied, as they have different targets and provided results which can be used in different ways. Prior to PLS analysis, data were mean centered and scaled to unit variance. Then, in order to meet PLS normality condition, Box-Cox transformation was applied to instrumental data when necessary (Box and Cox, 1964). PLS-1 models can be validated by a re-sampling leave-one-out method (Esbensen, 2009). Thus, in PLS-1 results, R^2 was measured on the set of data used to implement the model, measuring how much the model fit the data. Q^2 , instead, was measured on the set of data used to validate the model, thus, it measured how much the model was effective to estimate prediction. Therefore, to show the efficacy of the implemented predictive models, the Q^2 for each model was reported.

Triangle data analysis was performed by FIZZ Calculation software (2.46A, Biosystemes, Couternon, France), PCA and PLS were performed by The Unscrambler v9.8 software (CAMO Software, Norway), while all the other statistical analyses were performed by STATISTICA 9.1 software (StatSoft Inc., U.S.A.).

3 Results

3.1 Method validation

246 As for the triangle test performed to evaluate the antioxidant solution effect on apples, no
247 significant differences were found in terms of sensory perception in any of the 3 comparisons
248 (Table 2). Moreover, chemical measurements on the same samples confirmed the results from
249 sensory tests, showing no differences in terms of titratable acidity nor soluble solid concentration
250 for any cultivar (Table 3).

251 As for the panel performance evaluation, in both seasons, the ANOVA showed a significant judge
252 effect for every attribute, as expected for sensory data, since each judge contributed differently in
253 describing sample variability. Judge x product interaction was also significant for every attribute in
254 both years. Although the judges provided different contributions, their evaluations were consistent
255 enough to allow discrimination between cultivars. Indeed, the product effect was significant in both
256 years, demonstrating that the method was discriminant even when used by different sensory panels.
257 The replicate effect was significant only for “graininess” in 2010 and for “flouriness” and
258 “astringency” in 2011. Judge x replicate interaction was significant for “yellow flesh”,
259 “fibrousness”, “graininess” ($p < 0.01$), and for “astringency” ($p < 0.001$) in 2011 only. Product x
260 replicate interaction was significant for “crunchiness”, “fibrousness” and “graininess” in 2010, and
261 for “sour taste” and “crunchiness” ($p < 0.01$) in 2011. A careful analysis showed that a few judges
262 had problems in their reproducibility on specific attributes (different judges for different attributes).
263 Thus, such punctual problems were not considered sufficient to exclude their data from the data-set,
264 as they did not affect the overall sensory data reliability. Indeed, replicate effect was found to be not
265 significant for any attribute when average panel data were considered.
266 Therefore, the attribute average scores were used for the following analyses.

267

268 **3.2 Apple profiling**

269

270 The first two principal components from PCA on sensory data explained 78% of total variance in
271 the dataset. In Fig. 1a, the first principal component is led by texture attributes, while the second is

272 related to external appearance and flavour properties. Score distribution in Fig. 1b shows that the
273 same cultivars analysed in both years were described in a consistent manner by the trained panel.
274 Floury and acid cultivars were located in the lower-right quadrant ('Renetta', 'Gloster'), while
275 grainy and sweet cultivars were in the upper-right quadrant ('Golden Delicious', 'Gala',
276 'Morgenduft', 'Rubens'). Hard, crunchy, and sour apples were in the lower-left quadrant ('Granny
277 Smith', 'Goldrush') and crunchy and sweet cultivars were instead in the upper-left quadrant of the
278 plot ('Fuji', 'Pinova', 'Modi'TM).

279 Pearson's correlation coefficients showed that "crunchiness", "hardness", and "fibrousness" were
280 negatively correlated with "graininess" and "flouriness" ($r < -0.86$; $p < 0.001$). No correlation
281 between "sweet taste" and "flouriness" or "graininess" was found. "Sweet taste" was slightly
282 correlated with "juiciness", for $r = 0.43$, $p = 0.01$. A negative correlation, even if not high, between
283 "sour taste" and "juiciness" was found ($r = 0.47$; $p = 0.01$). "Sour taste" was, instead, linearly
284 correlated with "astringency", for $r = 0.81$, $p < 0.001$.

285 Mean values and standard deviation for instrumental evaluation are reported in supplementary data
286 Table S1. One-way ANOVA on instrumental data showed significant differences between the
287 cultivars. *P*-values were lower than 0.001 for all performed instrumental measurements, and for the
288 16 mechanical and acoustic parameters proposed in the method developed by Costa et al. (2011) for
289 apple texture analysis.

290 Correlation among the different instrumental parameters showed that textural mechanical
291 parameters were correlated with acoustic parameters with Pearson's correlation coefficient ranging
292 between 0.42 ($p < 0.05$) and 0.91 ($p < 0.001$; Table 6). The acoustic parameter AUX1 also showed a
293 slightly positive correlation with percentage of extractable juice ($r = 0.52$; $p = 0.004$). A slightly
294 negative correlation was found, instead, between percentage of extractable juice and titratable
295 acidity ($r = -0.53$; $p = 0.003$). The %SSC was positively correlated with the percentage of dry
296 matter ($r = 0.51$, $p = 0.05$).

297

298 3.3 *Sensory-instrumental relationship*

299

300 Fig. 2 shows the x and y loadings from PLS-2 analysis, with both instrumental mechanical-acoustic
301 and sensory texture properties defining the first principal component, while chemical and sensory
302 taste properties characterised the second one. Texture sensory attributes appeared to be related to
303 mechanical and acoustic texture parameters. “Juiciness”, instead, was less correlated to the texture
304 analyser data, but strongly related to the % of extractable juice, and “sour taste” was highly related
305 to the titratable acidity. “Sweet taste”, instead, could not be linked to %SSC. “Yellow flesh”
306 intensity was positively related to the b* measurement. Interestingly, the “sweet taste” attribute also
307 appeared to be related to the colorimeter data (Fig. 2).

308 Finally, such observations were considered as the starting point for the development of predictive
309 models for each sensory attribute.

310

311 3.4 *Predictive models*

312

313 The sensory and instrumental dataset was subjected to PLS-1 analyses, in order to estimate the best
314 prediction model for each sensory attribute. In table 7, PLS-1 models and relative Q^2 for each
315 sensory attribute using different series of instrumental data are reported. For the prediction of taste
316 sensory attributes, Box-Cox transformation of the instrumental data was necessary to meet the
317 normal distribution requirement of PLS method.

318 For each sensory attribute, a model using instrumental parameters corresponding to its specific
319 sensory description was first developed. However, a combination of different instrumental
320 variables, indirectly related to sensory attributes, was used to achieve better models. Thus in table 7,
321 only the best prediction model for each attribute is reported. The models using chemical and
322 colorimeter data (“Colour + Chemical”) were developed based on the 2011 dataset only, because

323 colorimeter measurements were included in the instrumental protocol only in the second
324 experimental year.

325

326 3.4.1 *Appearance attributes*

327 After the addition of colorimetric measurements to the instrumental analyses in 2011, an effective
328 prediction of flesh colour (green and yellow) was found with better results for “yellow flesh” than
329 “green flesh”. Interestingly, in both cases the best models were obtained using chemical parameters
330 (i.e., %SSC and titratable acidity) rather than colorimetric data alone (Table 7).

331

332 3.4.2 *Texture attributes*

333 The different instrumental parameters defined to assess fruit texture were adequate to efficiently
334 predict all the texture sensory attributes (with $Q^2 \geq 0.77$), with the exception of “juiciness” ($Q^2 =$
335 0.81, Table 7). The mechanical parameters from the texture analyser appeared to have different
336 contributions for the prediction of different sensory texture attributes. In general, each parameter
337 contributed significantly to at least one predictive model. The best model for the “juiciness”
338 attribute was instead achieved by using the whole instrumental dataset. Significant variables in the
339 prediction model were texture analyser data, % of extractable juice, L* parameter from colorimeter
340 analyses, and titratable acidity.

341

342 3.4.3 *Flavour attributes*

343 As already observed in the PLS-2 plot discussed in paragraph 3.3, “sweet taste” attribute was
344 related to colorimetric data. Actually, the best predictions for taste attributes were obtained using a
345 model based on chemical and colorimetric parameters, available for only the 2011 data-set, giving a
346 Q^2 value of 0.82 and 0.89 for “sweet taste” and “sour taste”, respectively (Table 7).

347

348 4 Discussion

349

350 **4.1 *The relationship within sensory data***

351

352 The sensory description of the considered apple cultivar selection showed that texture was
353 responsible for most of the variance existing among the samples, in agreement with the findings
354 presented by other authors (Mehinagic et al. 2003; Echeverría et al., 2008). The relationships
355 between the different sensory attributes can be deduced from the loading projection depicted in Fig.
356 1a and confirmed by Pearson's correlation coefficients: "flouriness" and "graininess" resulted
357 negatively correlated to "hardness", "crunchiness" and "fibrousness". The intercorrelation between
358 the different texture attributes demonstrated the multi-parameter nature of texture (Szczesniak,
359 2002).

360 The relationship between juiciness-mealiness and sweetness has been thoroughly investigated in the
361 literature, starting from the hypothesis that sweetness perception is influenced by texture properties
362 (thus, it could depend directly on juiciness or mealiness intensity). Thus, the relation between
363 "sweet taste" and texture attributes was studied. However, in our data no correlation between
364 "sweet taste" and "flouriness" or "graininess" and only a slightly link with "juiciness" were found.
365 Echeverría et al. (2008) highlighted a relationship between sweetness and mealiness which was
366 clear only after applying a non-negligible rotation factor in their Generalized Procrustes Analysis.
367 The rotation made high mealiness values match with low sweetness values. This effect was not
368 supported by a linear correlation between the two factors ($r = -0.15$; Echeverría et al., 2008). Harker
369 et al. (2006) supposed that sweetness perception could depend on the degree of breakdown of apple
370 flesh during chewing, rather than on differences in sugar and acid content. Therefore, the authors
371 suggested the existence of a relationship between juice release and sweetness perception. Their
372 results, however, do not clearly support this hypothesis. Moreover, Echeverría et al. (2008)
373 highlighted a low consensus in their sensory panel for sweetness attribute. Similar results were also
374 obtained in this investigation: there was little agreement among judges for the "sweet taste"

375 attribute, with the sensory panel showing average Pearson's correlation coefficients between each
376 judge and the mean panellist of 0.54 and 0.57 in 2010 and 2011, respectively, while the average
377 correlation for every attribute was higher than 0.7 in both years. This could be explained by a
378 possible interference by other sensory properties on sweetness perception, even if no clear evidence
379 of such relation exists in our results.

380

381 ***4.2 The relationship within instrumental data***

382

383 The correlation between mechanical and acoustic parameters confirmed the results previously
384 reported by Costa et al. (2011), thus validating the strict relation existing between structural
385 properties and acoustic response in apple (Vincent, 1998). Moreover, the correlation between
386 acoustic parameters and % of extractable juice can be explained by considering that the typical
387 “crispy” sound is due to a high internal turgor pressure and to the integrity of the cell wall structure.
388 Upon compression, the breakdown of this polysaccharide architecture releases the pressure together
389 with the internal compartmented liquid content (Duizer et al., 2001).

390 Finally, a slight correlation between %SSC and % dry matter was found, which could be explained
391 as %SSC is the result of the starch solubilisation process occurring during ripening (McGlone et al.,
392 2003; Palmer et al., 2010).

393

394 ***4.3 The relationship between sensory and instrumental data***

395

396 The best prediction models were developed for apple texture attributes. Other authors found good
397 correlations between the puncture test and sensory texture attributes as evaluated by a trained panel
398 (Harker et al., 2002a; Chauvin et al., 2010; Guerra et al., 2010). Our results confirmed that the
399 proposed texture analysis is an effective method to collect information about mechanical and
400 acoustic properties expressed by apple tissues during consumption; efficient models were developed

401 to predict sensory perception of apple texture properties on the basis of texture analyser data (Table
402 7). Moreover, our datasets include acoustic information not considered in many of the previous
403 studies. Zdunek et al. (2010) developed a similar tool for apple texture analysis, using a contact
404 acoustic emission detector, related to penetrometric equipment, to record the acoustic response of
405 apples during compression. They found a strong positive correlation between crispness,
406 crunchiness, and acoustic parameters (number of acoustic events and mean acoustic event
407 amplitude), with a Pearson's correlation coefficient varying from 0.6 to 0.9. However, in their
408 investigation the variability observed was due to different fruit from the same batch being used for
409 sensory and instrumental evaluations (Zdunek et al., 2010). A similar limitation was also observed
410 in De Belie et al. (2002), which compared sensory crispness with the recorded sound produced by
411 Royal Gala apples during biting. The authors underline that instrumental recordings were made on a
412 subject chewing a piece of apple, while sensory scores were provided by different volunteers of a
413 trained sensory panel on different pieces from the same apples. The best correlation they reported
414 was $r = 0.65$, because of differences in oral cavity shape and force-deformation patterns operated by
415 the front teeth of the different subjects (De Belie et al., 2002). Ioannides et al. (2009) also provide
416 similar results, by the use of an electromyography of masticatory muscles on subjects evaluating
417 texture attributes of apples. In their work, the main source of variability was attributed to the
418 subjects. Moreover, the authors found another source of variability of psychological origin in which
419 subjects tended to chew differently when asked to score specific sensory attributes (Ioannides et al.,
420 2009).

421 The advantage of our texture method, compared to the other studies discussed here, is the
422 possibility to process samples from the same single apple, with equal shape and size, available for
423 both sensory and instrumental measurements. The flesh cylinders cut from the same fruit were used
424 for sensory and instrumental measurements, in order to truly compare these two data types.
425 Moreover, the texture measurements guarantee the standardisation of the compression method, due

426 to a specified probe speed and percentage of strain during the test. With these settings, the different
427 acoustic responses can only refer to the actual differences between the samples.

428 The acoustic parameters generated by the texture analyser turned out to be significant variables used
429 in the PLS-1 model for the prediction of “crunchiness”, but also for the other texture sensory
430 attributes. This could suggest that the sensory perception of “hardness”, “flouriness”, “fibrousness”,
431 “graininess”, and “juiciness” of apples is not only related to tactile and mechanical properties of
432 apple flesh, but can also be influenced by acoustic information (Demattè et al., 2013). The reason
433 for the apparent relation observed in the PLS-1 models could be referred to the correlation between
434 mechanical and acoustic properties. The sound emission, related to the expansion of the cell liquid
435 content, is possible only if strong linkages in the middle lamella exist, so that the cell walls break
436 rather than slipper against each other (Longhi et al., 2013). This means that sound emission is only
437 possible when the fruit flesh is characterised by specific mechanical properties, which are therefore
438 important for acoustic perception during biting and chewing (Vincent, 1998; Duizer, 2001). This
439 relation was also observed for the “crunchiness” prediction, in which the model based on 16
440 mechanical and acoustic parameters performed better than the prediction model based only on four
441 acoustic variables, increasing from $Q^2 = 0.69$ to 0.85 (Fig. 3).

442 Among the texture attributes, juiciness was the only one which needed more data than the texture
443 analyser parameters for the development of an effective prediction model. The significance of
444 titratable acidity in the model confirm the correlation observed between “sour taste” and “juiciness”
445 attribute. It seems to be not only a relation between tastants and juiciness perception, since
446 instrumental measurements also confirmed the existence of a relationship. Moreover, the negative
447 trend observed between the sensory and the instrumental parameters indicated that the higher the
448 acid concentrations, the lower the “juiciness” score. To our knowledge, this is the first study where
449 this relation was highlighted.

450 For other sensory attributes, interesting relationships with instrumental parameters were found, as in
451 the case of “yellow flesh”: b^* parameter increases as the light wavelength passes from blue to

452 yellow (Schanda, 2007), thus a direct relationship between the two was expected. Instead,
453 unexpected relations were of fundamental importance for the definition of the best prediction
454 models, such as the relationship between “sweet taste” and colorimeter data. In the case of flesh
455 colour, a combination of colorimetric and chemical data seems to provide better information for
456 effective prediction (Table 7). We hypothesise an indirect relation between colour and carotenoids
457 or other chemical compounds, as they change during ripening. Flesh colour tends to go from green
458 to yellow as fruit ripens, and pigment content changes from a high concentration of chlorophyll to a
459 high concentration of carotenoids (Ampomah-Dwamena et al., 2012). The ripening process also
460 involves chemical compounds, with a reduction of acid content and an increase in SSC/titratable
461 acidity ratio (Jan and Rab, 2012). During post-harvest, even in the same storage condition, apple
462 cultivars do not follow the same ripening trends, because of different genetic factors (Jobling and
463 McGlasson, 1995; Johnston et al., 2002; Costa et al., 2010). Therefore, the ripening process during
464 two months of storage was not the same for all the cultivars here considered, as already observed in
465 Corollaro et al. (2013). This is why additional information about chemical composition helped to
466 better predict flesh colour sensory perception.

467 The relation between flesh colour and chemical composition was also observed for the prediction of
468 taste attributes. The best prediction model, indeed, was obtained by using a combination of
469 chemical and colorimeter data. This suggests a relationship between the flesh colour and the acidity
470 or sweetness perception, which could be explained by a multisensory interaction. Due to the
471 changes in the chemical composition during ripening, it is easy for the consumer to expect a sour
472 taste for an apple showing a green flesh, and vice versa. Nevertheless, different apple cultivars show
473 different flesh colours depending on their genetic characteristics. A difference in flesh colour might
474 be indirectly related to acid or sweet taste expectations, thus, to a bias in the sweet or sour taste
475 intensity evaluation, even when evaluated by a trained panel, without any relationship with the
476 actual ripeness of the fruit. By considering these observations, we can explain the difficulties met
477 by most of the authors in predicting sweetness by instrumental measures, who usually suggest a

relationship between sweet perception and texture properties (Plotto et al., 1999; Harker et al., 2002b; Oraguzie et al., 2009). We suggest that the effect of expectations in terms of taste, as affected by apple flesh colour, could be the reason for these difficulties. Of course, the evaluation of sweet and sour taste in our samples would have been more objective if the samples were evaluated under red light. However, our results better reflects the real consumption condition and what consumers perceive during apple tasting. Moreover, they permitted a good prediction of sweet taste perception by instrumental characterisation.

It was not possible to define a reliable prediction model for the “astringency” attribute, 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, from our results, “astringency” seems to be partially predicted by the complete dataset. This can be explained by the general correlation with the stage of ripening, since PA concentration tends to decrease with the progression of fruit ripening (Henry-Kirk et al., 2012).

5 Conclusion

Our combined sensory-instrumental approach allowed the description of a large sample of apple cultivars in an effective manner. Their perceptible quality was objectively measured and the relationships among physical, chemical, and sensory properties were highlighted. Finally, effective predictive models were estimated for: a) flesh appearance sensory properties, using colorimeter measurements; b) texture attributes, by means of the innovative texture analyser protocol; c) and taste properties, through a combination of chemical and colorimeter data. The study was carried out over two consecutive years with good results and comparable sensory descriptions of the same cultivars analysed during both years, confirming that the method was correctly implemented.

The proposed combined sensory-instrumental tool can be used as a valid method to outline/describe sensory properties of apple. It can be advisable when sensory analysis is not feasible because of the

limits in using humans, or because of scarcity of fruit material - for instance, in the case of large sample sets needed by genomic investigations. In latter case, in particular, sensory analysis might allow to define proper prediction models, which can be further applied on large apple samples to estimate their sensory profile by a rapid instrumental analysis.

Further research will look at simplifying procedures and better selecting the most important instrumental variables, making the method easier to apply in practice.

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645 **Table 1:** Apple cultivars analysed during 2010 and 2011 seasons. “Code” refers to the codes
646 used in Figs. 1, 2, and 3; “0” and “1” following the codes refer to 2010 and 2011, respectively.
647 Where present, the letters “a”, “b”, “c”, and “d” refer to the different orchards for cultivars having
648 more than one origin, as specified in column “Location”. The specific clones employed in this work
649 are reported between brackets.
650

Cultivar	Code	Location	Harvest	Analysis	Fruit weight ^a
Braeburn	BRN_0a	Giaroni	30/09/2010	26/11/2010	210.4
Braeburn	BRN_0b	Maso Part	01/10/2010	30/11/2010	238.6
Braeburn	BRN_1	Maso Part	27/09/2011	07/12/2011	252
Crimson Crisp™	CRI_1	Maso Maiano	18/08/2011	19/10/2011	223.5
Cripps Pink	PIN_0a	Giaroni	20/10/2010	22/12/2010	201.3
Cripps Pink	PIN_0b	Maso Part	26/10/2010	22/12/2010	188
Cripps Pink	PIN_1	Maso Part	24/10/2011	21/12/2011	209.3
Dalnette	DAL_1	Maso Part	11/10/2011	14/12/2011	224.1
Delblush	DLB_1	Maso Part	22/09/2011	25/11/2011	261.5
Delectable	DEL_0	Giaroni	04/08/2010	06/10/2010	166.1
Florina	FLO_0	Laimburg	14/09/2010	10/11/2010	246.3
Fuji (Kiku 8)	FUJ_0a	Giaroni	01/10/2010	30/11/2010	267.8
Fuji (Kiku 8)	FUJ_0b	Maso Part	05/10/2010	07/12/2010	270.9
Fuji (Kiku 8)	FUJ_1	Maso Part	06/10/2011	07/12/2011	270
Gala (Schniga)	GAL_0	Giaroni	23/08/2010	20/10/2010	169.6
Gala (Schniga)	GAL_1	Maso Part	09/08/2011	12/10/2011	185.7
Gloster	GLO_0	Giaroni	14/09/2010	10/11/2010	249.6
Gloster	GLO_1	Maso Part	08/09/2011	09/11/2011	257.2
Goldrush™	GDR_0	Giaroni	30/10/2010	22/12/2010	270.9
Goldrush™	GDR_1	Maso Part	24/10/2011	16/12/2011	280.7
Golden Delicious (B)	GOL_0a	Giaroni	16/09/2010	17/11/2010	222.1
Golden Delicious (B)	GOL_0b	Maso Part	24/09/2010	24/11/2010	248.4
Golden Delicious (B)	GOL_1	Maso Part	12/09/2011	11/11/2011	255.1
Granny Smith	GRA_0a	Giaroni	30/09/2010	26/11/2010	226.7
Granny Smith	GRA_0b	Maso Part	30/09/2010	30/11/2010	257.4
Granny Smith	GRA_1	Maso Part	22/09/2011	25/11/2011	268.1
Idared	IDA_0	Giaroni	30/09/2010	26/11/2010	250.4
Jazz™	JAZ_1	Laimburg	27/09/2011	30/11/2011	213.8
Kanzi™	KAN_1	Laimburg	16/09/2011	23/11/2011	216.4
Modi™	MOD_0	Giaroni	07/09/2010	03/11/2010	174.5
Modi™	MOD_1	Maso Part	01/09/2011	02/11/2011	226.5
Morgenduft (Dallago)	MOR_0	Maso Part	01/10/2010	07/12/2010	264.7
Morgenduft (Dallago)	MOR_1	Maso Part	27/09/2011	30/11/2011	305.5
Pilot	PIL_0	Giaroni	15/09/2010	17/11/2010	225.3
Pilot	PIL_1	Maso Part	08/09/2011	09/11/2011	205.8
Pinova	PNV_0	Maso Maiano	28/09/2010	24/11/2010	221.8
Pinova	PNV_1	Maso Part	13/09/2011	16/11/2011	231.7
Pinova (Roho)	RHO_1	Maso Maiano	15/09/2011	23/11/2011	222.2
Red Chief	RCF_0	Giaroni	07/09/2010	03/11/2010	268.7
Red Chief	RCF_1	Maso Part	31/08/2011	26/10/2011	299.3
Red Delicious	RED_0	Maso Maiano	20/09/2010	17/11/2010	222.3
Red Delicious	RED_1	Maso Part	31/08/2011	26/10/2011	277.7
Red Spur (Jeromine)	JER_1	Maso Part	31/08/2011	02/11/2011	301.4
Renetta Bianca	RNB_0a	Giaroni	07/09/2010	03/11/2010	318.8
Renetta Bianca	RNB_0c	Maso Maiano	20/09/2010	19/11/2010	257.9
Renetta Bianca	RNB_1b	Maso Part	31/08/2011	26/10/2011	296.7
Renetta Bianca	RNB_1c	Maso Maiano	13/09/2011	11/11/2011	256.9

Renetta Grigia	RNG_1b	Maso Part	31/08/2011	02/11/2011	310.1
Renetta Grigia	RNG_1c	Maso Maiano	13/09/2011	16/11/2011	282.3
Rubens™	RUB_0	Maso Maiano	21/09/2010	19/11/2010	191.4
Rubens™	RUB_1	Maso Part	08/09/2011	09/11/2011	243.8
Stayman	STY_0	Maso Part	04/10/2010	07/12/2010	289.9
Stayman	STY_1	Maso Part	22/09/2011	30/11/2011	309.2
Topaz	TOP_0	Maso Maiano	28/09/2010	24/11/2010	236.2
Topaz	TOP_1	Maso Part	15/09/2011	23/11/2011	250.1

a: average value from 20 fruit, expressed as grams.

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653 **Table 2:** Results of triangle tests performed on 3 apple cultivars: for each test total and correct
654 responses given by the panel and relative *p*-values are reported.
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	Total responses	Correct responses	<i>p</i>-value
Test 1: Granny Smith	30	14	0.0898
Test 2: Fuji	30	10	0.5683
Test 3: Cripps Pink	30	12	0.2761

658 **Table 3:** Mean values for titratable acidity and %SSC measured on apples (n = 6) from the
 659 triangle test performed to evaluate the antioxidant solution effect on apples. *P*-values from one-way
 660 ANOVA performed on treated and untreated samples are reported.
 661

		Treated Antioxidant		Treated No Antioxidant		<i>p</i> -value
		Mean	Stand. Dev.	Mean	Stand. Dev.	
Test 1: Granny Smith	Titratable acidity ^a	11.7	2.1	11.6	1.6	0.513
	%SSC	10.6	0.7	11.7	1.2	0.232
Test 2: Fuji	Titratable acidity ^a	4.8	1.3	4.5	1.1	0.758
	%SSC	14.9	1.0	14.1	0.5	0.288
Test 3: Cripps Pink	Titratable acidity ^a	6.5	0.3	6.2	1.0	0.646
	%SSC	12.4	0.7	11.9	0.3	0.312

662 a: expressed as malic acid equivalents in 100g juice.

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Table 4: Sensory lexicon used by the sensory panels.

Category	Attribute	Definition
Appearance	Green flesh	Flesh green depth
Appearance	Yellow flesh	Flesh yellow depth
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)

665 **Table 5:** Mechanical and acoustic parameters with respective code and description used for
666 mechanical and acoustic profiling.
667

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

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Table 6: Pearson's correlation coefficients calculated between instrumental parameters.

	%SS C	Titr. Acidity	% juice	% dry matter	L*	a*	b*	F1	F2	F3	FP	A	FLD	Y	F4	F1- F3	F1/F3	P/D	LD/D	AUX P	AUX 1	AUX 2
%SSC	-																					
Titr. Acidity	0.36	-																				
% juice	-0.14	-0.53	-																			
% dry matter	0.51	0.14	0.03	-																		
L*	0.35	0.24	-0.26	0.42	-																	
a*	0.12	0.02	-0.13	-0.08	-0.25	-																
b*	0.02	-0.48	0.33	-0.08	-0.31	0.43	-															
F1	0.16	0.25	0.14	0.13	-0.29	0.02	0.00	-														
F2	0.21	0.15	0.19	0.15	-0.34	0.06	0.06	0.96	-													
F3	0.27	0.17	0.27	0.20	-0.20	0.01	0.09	0.94	0.96	-												
FP	0.05	-0.17	0.53	0.12	-0.46	-0.16	0.05	0.45	0.55	0.52	-											
A	0.22	0.16	0.21	0.15	-0.34	0.05	0.06	0.97	1.00	0.96	0.57	-										
FLD	0.05	-0.01	0.25	0.02	-0.56	0.05	0.09	0.81	0.88	0.78	0.73	0.88	-									
Y	0.18	0.27	0.04	0.15	-0.26	0.05	-0.01	0.92	0.90	0.83	0.47	0.90	0.84	-								
F4	0.22	0.16	0.21	0.15	-0.34	0.06	0.06	0.97	1.00	0.96	0.57	1.00	0.88	0.90	-							
F1-F3	-0.38	0.10	-0.42	-0.27	-0.11	0.03	-0.25	-0.32	-0.46	-0.62	-0.41	-0.46	-0.30	-0.20	-0.46	-						
F1/F3	-0.22	-0.03	-0.56	-0.24	0.08	0.09	-0.17	-0.54	-0.56	-0.73	-0.56	-0.58	-0.43	-0.38	-0.58	0.78	-					
P/D	0.10	-0.19	0.65	0.19	-0.34	-0.17	0.08	0.52	0.60	0.64	0.93	0.62	0.66	0.44	0.62	-0.58	-0.75	-				
LD/D	0.13	-0.03	0.44	0.17	-0.15	0.00	0.16	0.77	0.75	0.85	0.37	0.76	0.58	0.56	0.76	-0.58	-0.75	0.62	-			
AUXP	0.01	-0.18	0.42	-0.02	-0.50	-0.07	-0.01	0.60	0.67	0.58	0.84	0.68	0.78	0.67	0.68	-0.23	-0.40	0.75	0.36	-		
AUX1	0.02	-0.14	0.52	0.05	-0.37	-0.07	-0.07	0.67	0.74	0.72	0.77	0.75	0.77	0.67	0.75	-0.44	-0.60	0.80	0.60	0.90	-	
AUX2	0.06	-0.25	0.67	0.15	-0.23	-0.10	0.03	0.52	0.61	0.66	0.79	0.63	0.59	0.42	0.63	-0.63	-0.76	0.91	0.65	0.76	0.89	-
AUXLD	0.03	-0.23	0.48	0.04	-0.47	-0.11	0.01	0.55	0.64	0.56	0.88	0.65	0.75	0.60	0.65	-0.30	-0.44	0.80	0.35	0.98	0.89	0.81

671

672 **Table 7:** PLS-1 models and relative Q^2 values, estimated for each sensory attribute, using
 673 different series of instrumental data for the development of the models. “Nr. components” column
 674 refers to the number of components used for achieving the best prediction model.
 675

Attribute (y)	Matrix of instrumental Data ^a (X)	Box Cox transformation ^b	PLS-1 model	Q^2	Nr. Components
Green flesh	Colour + Chemical	NT	$y = 0.4339X + 8.6972$	0.4911	1
Yellow flesh	Colour + Chemical	NT	$y = 0.8629X + 5.6267$	0.9019	2
Hardness	TA-XT	NT	$y = 0.8624X + 5.5972$	0.8770	1
Juiciness	All	NT	$y = 0.7896X + 9.8693$	0.8115	2
Crunchiness	TA-XT	NT	$y = 0.8455X + 6.7400$	0.8534	1
Flouriness	TA-XT	NT	$y = 0.7838X + 6.7745$	0.7867	2
Fibrousness	TA-XT	NT	$y = 0.7919X + 6.9158$	0.8003	1
Graininess	TA-XT	NT	$y = 0.7771X + 8.0034$	0.7696	2
Sweet taste	Colour + Chemical	T	$y = 0.8352X + 6.9063$	0.8184	3
Sour taste	Colour + Chemical	T	$y = 0.8647X + 4.8504$	0.8876	2
Astringency	All	NT	$y = 0.6109X + 8.3952$	0.5280	5

676 a: Colour + Chemical = L^*a^*b , %SSC and titratable acidity data; TA-XT = mechanical and acoustic texture analyser data; All = entire instrumental
 677 data-set
 678 b: T = transformed data; NT = untransformed data

679 **Figure captions**

680

681 **Fig. 1:** Loadings (a) and scores (b) plots from Principal Component Analysis (PCA) on sensory
682 data-set. For apple products coding, see “Code” column in Table 1.

683

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

688

689 **Fig. 3:** Predicted vs. measured plot from PLS-1 model developed for “crunchiness” sensory
690 attribute by means of acoustic and mechanical data from texture analyser analysis.
691 Predicted and validated interpolation lines are shown as dotted (grey) and continuous
692 (black) lines, respectively. Slope, offset, Root Mean Square Error (RMSE), are reported
693 for both. R^2 and Q^2 are also reported.

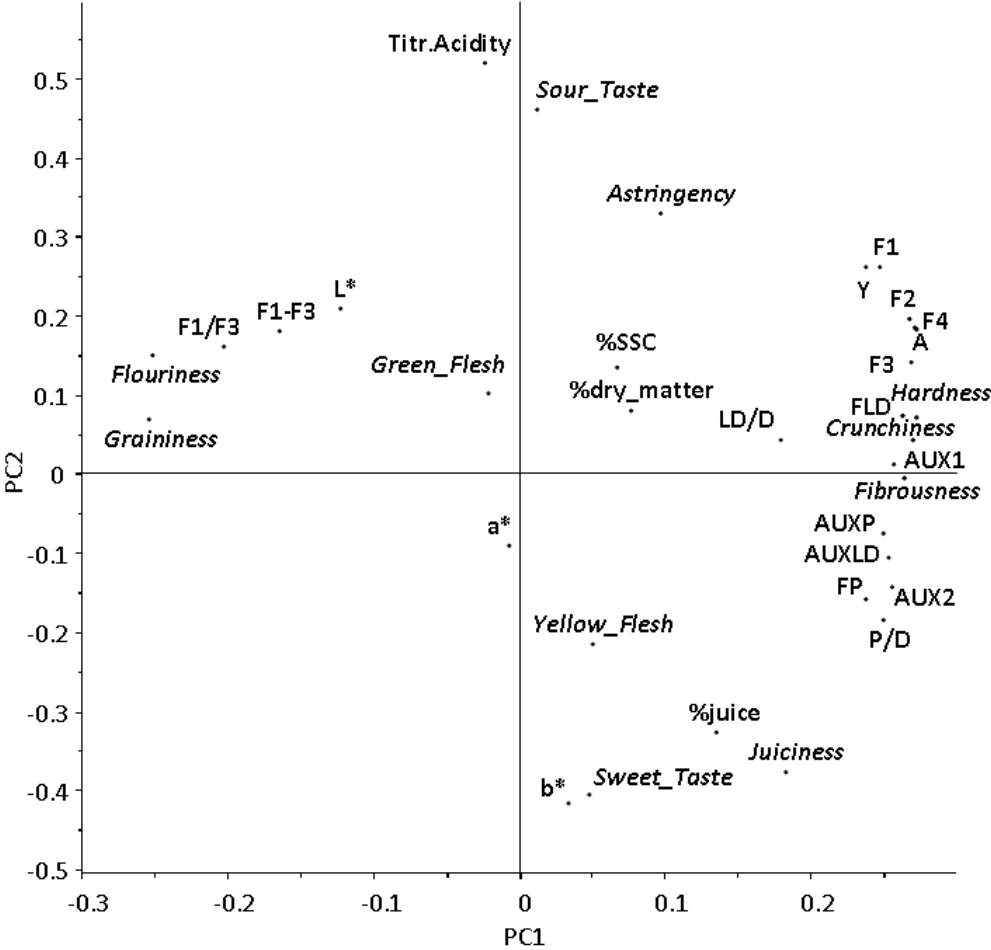
695



700

701 **Fig. 2**

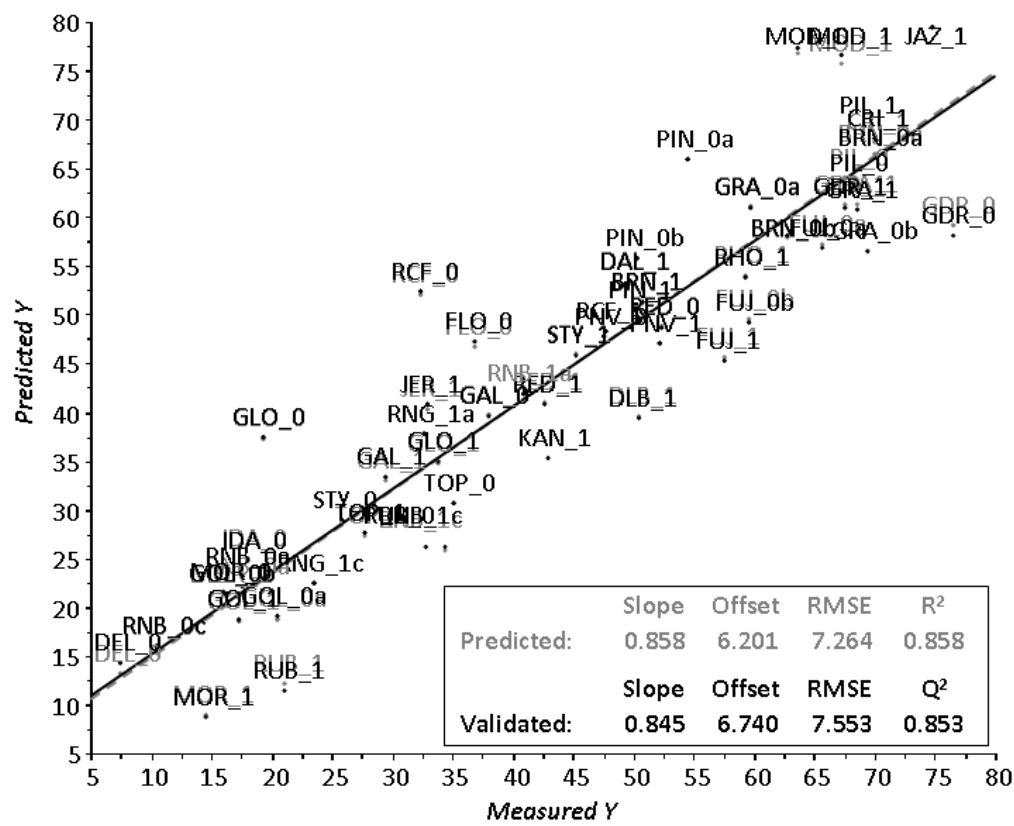
702



703

704 Fig. 3

705



706

707