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Chemical compounds and sensory assessment of kiwifruit (*Actinidia chinensis* (Planch.) var. *chinensis*): electrochemical and multivariate analyses

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This study reports the analytical and sensory analyses made on selected kiwifruit genotypes (*Actinidia chinensis* (Planch.) var. *chinensis*) produced from seeds of fruit gathered in the Guangxi region of the People's Republic of China. The analytical measurements of some soluble sugars, such as glucose and fructose, and non-volatile acids, such as malic and ascorbic acid, were carried out using innovative analytical procedures based on fast and selective devices that require very little or no sample treatment. The multivariate techniques, Principal Component Analysis and Cluster Analysis, useful when many variables are involved, allowed the classification of kiwifruit genotypes according to sugar and non-volatile acid contents and sensory properties. Citric acid, the major organic acid, ranged from 0.8 to 1.8 g per 100 g of fresh weight and malic acid content was 0.1–0.5 g per 100 g of fresh weight. The levels of fructose and glucose (present in approximately equal amounts in most of the genotypes analysed), were higher than that of sucrose in almost all the genotypes. Ascorbic acid content in kiwifruit samples from genotypes of *Actinidia chinensis* (Planch.) var. *chinensis* was higher than the typical mean content in *Actinidia chinensis* var. *deliciosa* (A Chev) cv Hayward. Sensory assessment showed that total fruit aroma and flavour were the best discriminating attributes and were highly correlated with overall quality. No consistent correlation was found between overall quality and the sensory attributes of sweetness, juiciness and firmness.

INTRODUCTION

The most widely grown kiwifruit crop is the *Actinidia chinensis* var. *deliciosa* (A Chev) cv Hayward. The commercial growing of this variety has spread to many countries because of its distinctive characteristics, such as size, uniformity of speckling and postharvest quality. Several investigations were carried out on changes in composition and the softening of *Actinidia chinensis* var. *deliciosa* cv Hayward during ripening (Mac Rae *et al.*, 1989) and postharvest treatments. Stec *et al.* (1989) found that parameters such as aroma intensity and acceptability, sweetness, acidity and ripe fruit flavour were significantly affected by the firmness of the fruit.

Kiwifruit contains significant amounts of vitamin C (L-ascorbic and L-dehydroascorbic acids) which is physiologically active in both forms (Wills & Greenfield, 1981). It contains more than the average amounts found in fruit such as grapefruit, oranges, strawberries and lemons and ten times as much as that found in apples and peaches (Beever & Hopkirk, 1990).

Inconsistencies in reported values for kiwifruit are largely owing to differences in varieties, degree of maturity, storage and method of analysis. For instance, the value range for 'Hayward' fruit was 37–200 mg per

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100 g of fresh weight (Selman, 1983; Lintas *et al.*, 1991). However, very little is known about the chemical composition, particularly ascorbic acid content and the sensory evaluation of other kiwifruit genotypes.

The objective of this work was to carry out analytical and sensory analyses in order to characterise selected genotypes of *Actinidia chinensis* (Planch.) var. *chinensis* originating from seeds of fruit gathered in the Guangxi region of the People's Republic of China that could contribute to improving the world's varieties.

The analytical measurements of ascorbic acid and certain soluble sugars and non-volatile acids were carried out using innovative analytical procedures based on fast and selective devices which require very little or no sample treatment (Mascini & Palleschi, 1989). In addition, the use of glucose, fructose, malic and ascorbic acid probes for the genetic improvement of the fruit produced good results.

MATERIALS AND METHODS

Kiwifruit

Twenty-three early-maturing (35–24 days before cv Hayward) kiwi genotypes (*Actinidia chinensis* (Planch.) var. *chinensis*) (Table 1) were obtained from the experimental orchards of the Istituto Sperimentale per la Frutticoltura (Fiorano, Rome, Italy). The trees were about 7 years old, grafted on the same rootstock, spaced at 4×2.5 m and trained on multi-wired fences. The fruit skin of some genotypes is thin and covered with very fine hairs that are largely deciduous, whereas others are completely smooth. The colour of the fruit flesh ranges from dark green to light yellow. The kiwis were hand-harvested at commercial ripeness and eating ripeness. Thirty fruits with the same firmness at touch were selected as representative samples and were equally divided into two groups for chemical and sensory analysis.

Chemicals

Glucose oxidase (EC 1.1.3.4 from *Apergillus niger*, type VII), fructose dehydrogenase (EC 1.1.99.11 from *Gluconobacter sp.*), L-malic acid (sodium salt) and malic enzyme (chicken liver; EC 1.1.1.40., 26 units mg^{-1}) were purchased from Sigma Chemical Co., St. Louis, MO, USA. Ascorbate oxidase (EC 1.10.3.3. from *Cucurbity sp.*) was obtained from Fluka Chemie, Buchs, Switzerland. Ferricyanide was from Boehringer, Mannheim, Germany and pyruvate oxidase from *Pediococcus sp.*, EC 1.2.3.3., 20.7 units mg^{-1}, Toyo Iozo, Shisuoka, Japan. A cellulose acetate membrane (100 Da nominal molecular weight cut-off) was prepared in the laboratory according to the Mascini and Mazzei (1987) procedure. A microporous polycarbonate membrane 0.03 μm was obtained from Nuclepore (Pleasanton, CA, USA), the Immobilon-AV affinity membrane (0.65 μm

Table 1. Kiwifruit genotypes (*Actinidia chinensis* (Planch.) var. *chinensis*) used in this study

Genotype[a]	Mean weight (g per fruit)				Ripening +/−Hayward (day)			
	93	94	95	96	93	94	95	96
ILPGRH3	54	56	40	44	−30	−14	−24	−26
G.CUO 2	52	42	38	50	−29	−2	−28	−25
G.CUO 6	40	60		60	−44	−10	−36	
G.ALL 1	54	53	25	54	−38	−21	−34	
G.ALL 3	67	71	48	52	−30	−21	−30	
G.ALL 18				65			−36	−27
G.ALL 20				64			−32	−32
GTH 4		100		56		−11		
M.CUO 5	32	33	40	55	−43	−13	−35	−36
M.CUO 10	40		30	42	−28		−32	−26
M.CUO 11	37	16	36	46	−34	−20	−28	−29
M.CUO 17	70	85	41	65	−28	4	−26	−20
MED 2		62	30	47		−23	−35	−27
MED 10	49		42	55	−35			−22
M.ALL 5	68	76	51	64	−29	−10	−26	−25
MTHPS 2	46	45	25	40	−38	−14	−29	
PIC 1	23		14	31	−38		−35	−22
PIC 9	27			20	−44		−32	
PIC 16	38	32	21	20	−39	−22	−25	
PIC 26	46			23	−39		−34	
PIC 36		22	20	35		−13	−34	
PLA 1	56	26	31	47	−29	−2	−28	−16
TGE 8			20	41				

[a]ILPGRH, very large, Hayward shape, G.CUO, large, heart-shaped, G.ALL, large, long and narrow; GTH, large, Hayward shape; M.CUO, medium-sized, heart-shaped; MED, medium-sized, Hayward shape; M.ALL, medium-sized, long and narrow; MTHPS, medium-sized, ellipsoidal; PIC, small; PLA, flat-shaped; TGE, Goering shape.

pore size, 125 μm thick) was from Millipore (Bedford, MA, USA) and the Pall Immunodyne immunoaffinity membrane was from Pall Corp. (Glen Cove, NY, USA).

Apparatus

Electrochemical measurements were carried out using an ABD (Amperometric Biosensor Detector) from Universal Sensors (Metaire, LA, USA). The probe used for batch analysis was a hydrogen peroxide electrode from Universal Sensors. Currents were recorded with a model 868 AMEL recorder (Milan, Italy).

Chemical analysis

Fifteen fruits from each genotype were equally divided into three replications. The fruits were peeled and ground to a puree in a Waring blender. The homogenate was filtered and the juice analysed. Glucose, malate and fructose determinations were carried out using electrochemical biosensors. The electrochemical transducer used to determine glucose and malic acid was an H_2O_2 platinum electrode maintained at +650 mV applied potential vs a silver/silver chloride cathode. As regards the ascorbate determinations, oxygen decrease, caused by enzymatic reaction, was determined by a Clark electrode (Pt cathode maintained at −680 mV vs a built-in silver/silver chloride reference electrode).

The reactions were as follows:

Glucose:

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

Malate:

$$\text{(a)L} - \text{Malate} + NADP^+ \xrightarrow{\text{Malic enzyme}} \text{Pyruvate} + NADPH + CO_2 + H^+$$

$$\text{(b)Pyruvate} + HPO_4^{2-} + O_2 \xrightarrow{\text{Pyruvate oxidase}} \text{Acetylphosphate} + H_2O_2 + CO_2$$

Ascorbate:

$$\text{L} - \text{Ascorbate} + 1/2O_2 \xrightarrow{\text{Ascorbate oxidase}} \text{Dehydroascorbate} + H_2O$$

The procedures for assembling the glucose, malate and ascorbate probes and the analyses of glucose, malate and ascorbate were the same as those used in previous studies (Mascini *et al.*, 1988; Matsumoto *et al.*, 1988; Palleschi *et al.*, 1989, 1990; Messia *et al.*, 1996).

The working electrode used to determine fructose was a platinum electrode maintained at +250 mV vs a built-in silver–silver chloride reference electrode.

The reaction was as follows:

$$\text{Fructose} + \text{Mediator}_{OX} \xrightarrow{\text{Fructose dehydrogenase}} \text{Ketofructose} + \text{Mediator}_{RID}$$

$$\text{Mediator}_{RID} \xrightarrow{\text{Electrode}} \text{Mediator}_{OX}$$

Measurements were carried out in a phosphate buffer 0.1 M pH 7.0, where potassium ferricyanide ($K_3Fe(CN)_6$), 1.5 mmol litre^{-1} was used as the mediator. The enzymes were immobilised on the membranes according to a procedure reported in the literature (Palleschi *et al.*, 1990; Messia *et al.*, 1996). Spectrophotometric determinations of citric acid and sucrose were carried out using Boeringher Mannheim kits n.139076 and n.716260 (Mannheim, Germany), respectively.

A special ISFET-based pH-probe from Sentron (pH-System 1001) Roden, The Netherlands, constructed for direct pH measurements in semi-solid matrices, was inserted into the fruits to measure the pH. The soluble solid was measured with an ATAGO refractometer at 20°C. The results are reported in Brix degrees at 20°C.

Sensory analysis

Sensory tests were carried out by a 10-member panel trained to assess colour on a 6-point category scale (green to yellow); acid character, sweetness, aroma/flavour intensity, firmness, juiciness and overall quality on a 9-point (nil to extreme) category scale. In each sensory session, six samples were distributed to the assessors in randomised order. Panellists received half a longitudinally cut fruit for each sample so there would be no variations between individual fruits. Each assessor gave three replicated judgements for a total of 15 fruits from each genotype. Evaluation took place in individual tasting booths at room temperature under cool white fluorescent light and each assessor was provided with a knife, spoon and a glass of rinsing water.

Statistical analysis

In order to analyse the results, the overall fruit means of the chemical data were calculated and the averages from both assessors and replicates were used for the sensory data. A multivariate approach was used for data interpretation. Chemical and sensory data were separately processed using the Principal Component Analysis (PCA) to determine which chemical/sensory parameters contributed most to drawing distinctions within the data sets. Cluster Analysis (CA) was used for the kiwi genotypes classification. The hierarchical centroid method was used for CA (Hair *et al.*, 1987). In order to measure similarities between the chemical variables and between the sensory attributes, the squared Euclidean distance between two cluster centroids was considered. For the normalised Euclidean distance, a threshold of

0.7 was chosen to locate the clusters (SAS, 1990). An SAS package (SAS, 1990) was used to process data.

RESULTS AND DISCUSSION

Analytical optimisation

Calibration curves of glucose, malate, fructose and ascorbate, carried out with electrochemical biosensors, led to the results reported in Table 2, which shows the lower and upper detection limits, the linearity range and the relative standard deviation calculated on three consecutive measurements at a fixed standard concentration and with selected samples of fruit. The probes gave good reproducible results. Calibrations were performed during fruit analysis and both stability and drift probes were evaluated. The sensitivity of the probes enabled a dilution of 1/500, 1/200, 1/1000 and 1/20 for glucose, malate, fructose and ascorbate, respectively.

The stability of the probes was good; probe drift, calculated over one day, gave a relative error of less than 1%, which is totally compatible with the accuracy of the analysis since the relative error calculated was 3–5%. Many analytical methods, based on different techniques, are reported in the literature for the quantitative determination of ascorbic acid (AOAC, 1980; Bajaj & Kaur, 1981; Oi-Wah Lau *et al.*, 1989; Graham & Annette, 1992; Kishida *et al.*, 1992; Nisperos-Carriedo *et al.*, 1992; Uchiyama *et al.*, 1991; Lorenti *et al.*, 1992; Vinci *et al.*, 1995) but some have drawbacks such as low sensitivity and lengthy procedures. The enzymatic method applied in this study is more accurate because of the easier and faster sample pre-treatment procedure used.

Soluble sugar and non-volatile acid composition

The sugar and non-volatile acid composition of kiwifruit genotypes is given in Tables 3 and 4. The main soluble sugars in kiwifruit (*Actinidia chinensis* var. *deliciosa*) are glucose and fructose, whereas sucrose is present in smaller amounts (Mac Rae *et al.*, 1989; Beever & Hopkirk, 1990; Lintas *et al.*, 1991). Fructose and glucose, present in approximately equal amounts in most of the genotypes analysed, were the major sugars found in all the genotypes except for M.CUO 5, where sucrose predominated.

Only citric and malic acid, the major acids in kiwifruit, were quantitatively analysed. Citric acid, the main organic acid component, ranged from 0.8 to 1.8 g per 100 g of fresh weight and malic acid from 0.1 to 0.5 g per 100 g of fresh weight. These values concur with previous studies (Mac Rae *et al.*, 1989; Beever & Hopkirk, 1990; Lintas *et al.*, 1991).

Figure 1 illustrates the correlations between the chemical variables and the first two dimensions using the PCA built on the normalised variables. The first two dimensions, significant at the cross-validation procedure (Wold, 1978), accounted for 70% of the total variance. The first dimension (46.1% explained variance) was closely and positively correlated with glucose, fructose, sucrose and pH while the organic acids, malic and citric, were positively correlated with the second dimension (23.9% explained variance). The correlation matrix of

Table 2. Analytical performance of biosensors

Sensor	Lower detection limit (mol litre^{-1})	Upper detection limit (mol litre^{-1})	Linearity range (mol litre^{-1})	Relative standard deviation (%)
Glucose	5.10^{-7}	2.10^{-3}	1.10^{-6}–1.10^{-3}	2.2
Malate	5.10^{-7}	1.10^{-3}	1.10^{-6}–5.10^{-4}	1.7
Fructose	1.10^{-6}	5.10^{-4}	5.10^{-6}–1.10^{-4}	3.4
Ascorbate	1.10^{-5}	1.10^{-3}	$2.5.10^{-5}$–$7.5.10^{-4}$	2.5

Table 3. pH, soluble sugar and non-volatile acid composition (g per 100 g FW) of fruits from 12 kiwifruit genotypes (*Actinidia chinensis* (Planch.) var. *chinensis*) at commercial ripeness

Genotype	pH	Fructose	Glucose	Sucrose[a]	Malic acid	Citric acid[a]
G.ALL 3	3.5	0.48	0.44	0.27	0.19	1.44
G.ALL 20	3.5	0.91	0.83	0.12	0.15	1.03
G.CUO 6	3.1	2.15	2.11	0.39	0.20	1.58
GTH4	3.5	0.53	0.51	0.09	0.27	1.10
M.CUO 17	3.1	0.35	0.33	0.04	0.23	1.20
MED 2	3.1	1.20	1.05	0.06	0.22	1.75
MTHPS 2	3.2	1.25	1.22	0.27	0.27	1.31
PIC 1	3.4	1.40	1.36	0.18	0.18	1.23
PIC 9	3.2	1.11	1.14	0.08	0.12	1.00
PIC 16	3.6	1.96	1.93	0.12	0.15	1.00
PIC 26	3.6	1.25	1.26	0.11	0.17	1.33
PIC 36	3.7	2.08	2.03	0.14	0.15	0.86

Values are the means of triplicate samples.
[a]Spectrophotometric measurements.

Table 4. pH, soluble sugar and non-volatile acid composition (g per 100 g FW) of fruits from 15 kiwifruit genotypes (*Actinidia chinensis* (Planch.) var. *chinensis*) at eating ripeness

Genotype	pH	Fructose	Glucose	Sucrose[a]	Malic acid	Citric acid[a]
ILPGRH 3	3.7	3.01	2.99	1.34	0.33	1.45
G.CUO 2	3.8	2.18	2.15	0.72	0.18	1.17
G.ALL 1	3.4	3.52	3.66	0.74	0.43	1.40
G.ALL 18	3.9	2.79	2.65	1.19	0.15	1.10
G.ALL 20	4.1	2.76	2.62	1.79	0.16	0.95
M.CUO 5	4.0	1.92	1.81	3.36	0.15	1.49
M.CUO 10	3.9	3.35	3.16	0.57	0.07	1.10
M.CUO 11	4.1	2.81	2.63	0.78	0.14	1.34
M.CUO 17	3.6	1.20	1.13	0.21	0.18	1.31
MED 2	3.5	2.30	2.20	1.96	0.34	1.60
MED 10	3.6	1.85	1.89	1.65	0.17	1.46
M.ALL 5	3.7	2.09	2.09	1.73	0.17	1.46
PIC 1	4.2	3.02	3.01	0.49	0.19	1.12
PLA 1	3.6	2.32	2.21	0.20	0.12	0.79
TGE 8	3.7	2.06	2.10	0.39	0.48	1.09

Values are the means of triplicate samples.
[a]Spectrophotometric measurements.

the analytical data only showed a significant linear correlation between glucose and fructose ($R = 0.996$).

Figure 2 shows the distribution of the co-ordinates of the kiwifruit genotypes on the first two principal components. The Cluster Analysis results are superimposed. Most of the kiwi genotypes harvested at commercial ripeness were clustered into two distinct groups (CL1 and CL2) located along the negative axis of the first dimension; M.CUO 17 was the only sample harvested at eating ripeness clustered in CL1. The largest cluster (CL1) consisted of nine genotypes; the characteristic of the kiwi fruits in this cluster was their low sugar content. The second cluster (CL2), comprised of only MED 2 and G.CUO 6, also harvested at commercial ripeness, differed from CL1 since these genotypes had higher levels of citric and malic acid.

Two clusters (CL3 and CL4) and three individual samples (G.ALL 1, TGE 8, M.CUO 5), including genotypes harvested at eating ripeness, had higher sugar contents but the CL3 had lower citric and malic acid contents. Two genotypes, PIC 16 and PIC 36, harvested at commercial ripeness, were classified under CL3. Four genotypes, MED 2, PIC1, G.ALL 20, and M.CUO 17, were analysed at both commercial and eating ripeness (linked by arrows). In Fig. 2 the PIC 1 and G.ALL 20 genotypes move from CL1 to CL3, while the MED 2 genotype moves from CL2 to CL4 as regards the time of harvesting (commercial and eating ripeness). Only M.CUO 17 remains classified in the same cluster (CL1) when analysed at eating ripeness.

By comparing Figs 1 and 2, the first component can be interpreted as a ripening dimension. By protracting ripening, the sugar and pH levels increase; however, malic

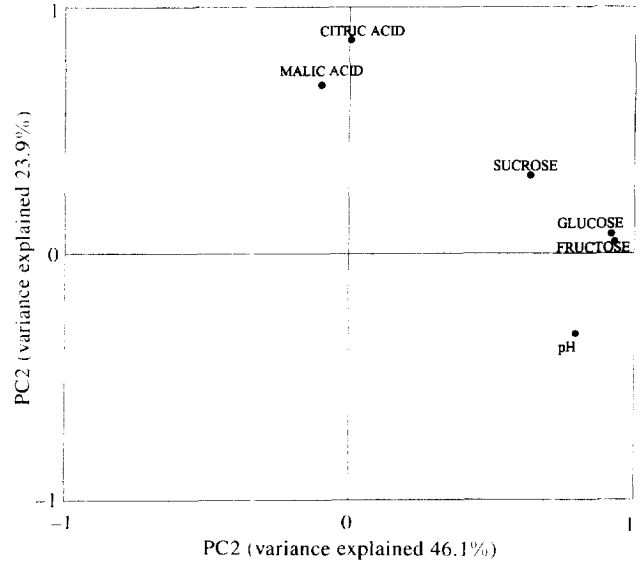

Fig. 1. Correlation plot of the chemical variables on the plane of the first two principal components.

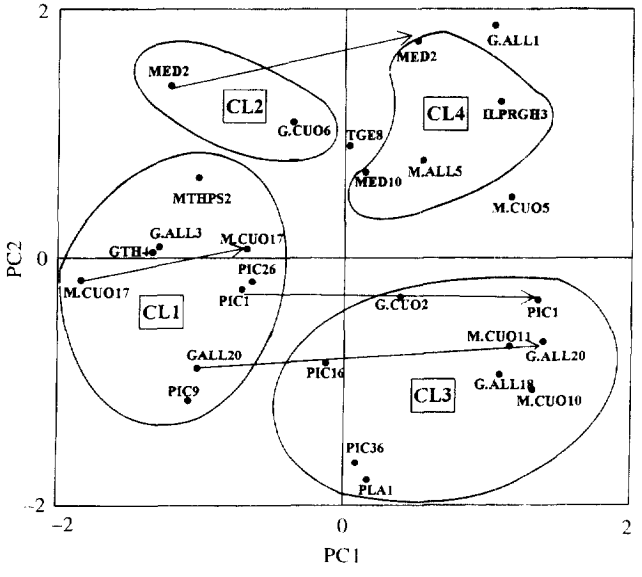

Fig. 2. Score plot of kiwi genotypes on the plane of the first two principal components of a PCA on the chemical variables. Samples are grouped into circles according to Cluster Analysis (CA).

and citric acid contents appear unchanged. The second dimension separates the kiwi genotypes on the basis of their organic acids (malic and citric) and shows that the fruits belonging to CL1 and CL3 are low-acid genotypes.

Ascorbic acid in kiwifruits

There are considerable variations in the reported ascorbic acid content of kiwifruit. This depends on the growing conditions, degree of ripeness, handling, storage and methods of analysis (Selman, 1983; Beever & Hopkirk, 1990). The ascorbic acid contents of seven kiwifruit genotypes of *Actinidia chinensis* (Planch.) var. *chinensis*, at different degrees of ripeness, are given in Fig. 3. It is interesting to note that the mean concentration, with the exclusion of M.ALL 5, is higher than the typical mean of ascorbic acid content of *Actinidia deliciosa* cultivars (80–120 mg per 100 g FW) as reported in the literature (Selman, 1983; Beever & Hopkirk, 1990; Lintas *et al.*, 1991). There can also be considerable intra-genotype variations in ascorbic acid content during ripening. Also, small decreases of ascorbic acid were observed in the M.CUO 5, MTHPS 2, PLA 1 and MED 2 genotypes, whereas for PIC1 and M.ALL 5, the ascorbic acid content was halved, 4.2–6 °Brix and 1.5–3.2 °Brix, respectively.

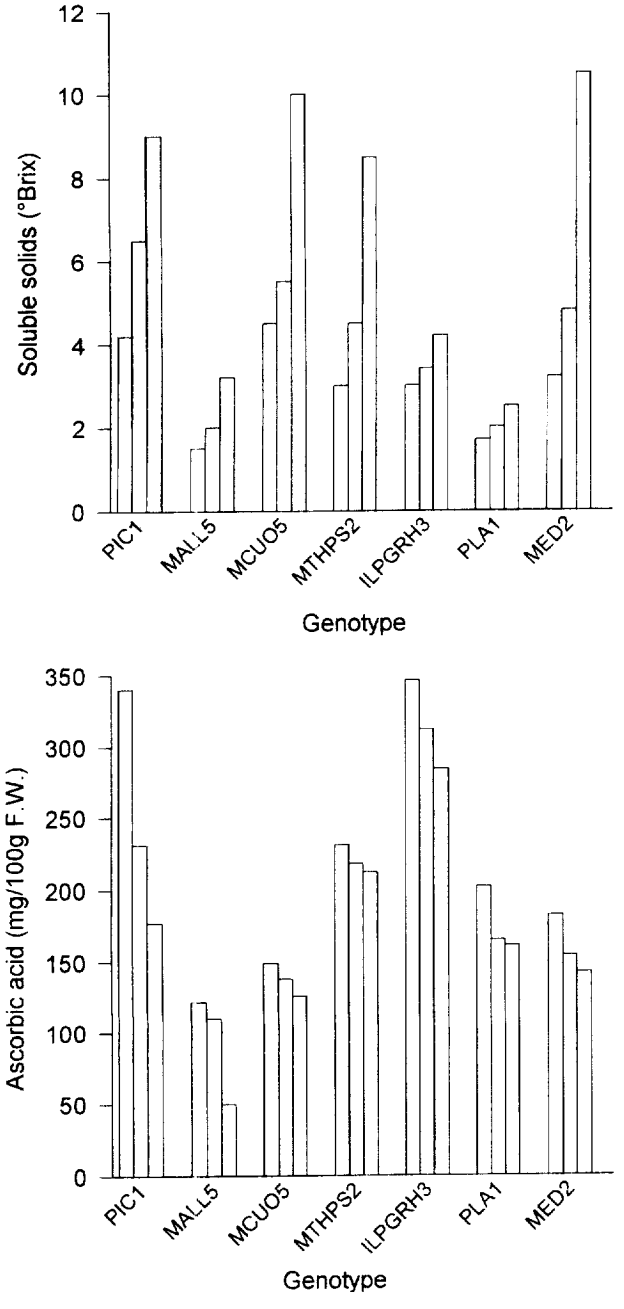

Fig. 3. Changes in soluble solids and ascorbic acid during ripening (9/19; 9/30; 10/9) of seven kiwifruit genotypes.

Sensory analysis

Figure 4 shows the correlation between the sensory variables and the first two principal components built on the normalised variables to correct differences in scale of the colour variable. As regards the sensory data, the first two dimensions accounted for 56.9% of the total variance (35.5 and 21.4%, respectively).

Total aroma and flavour intensity, sweetness and juiciness and overall quality were positively correlated, whereas sourness and firmness were negatively correlated with the first dimension. In particular, high variance in sourness and sweetness was explained on both dimensions and they were inversely correlated ($R = 0.70$, $P < 0.001$). Colour was clearly and positively correlated with the second dimension.

Fruit flavour plays a major role in the assessment of sensory quality: a most significant linear correlation was found between flavour and overall quality ($R = 0.91$, $P < 0.001$) and both were closely correlated with the first dimension. In Fig. 4, fruit aroma/flavour appears to be more intense in the softer fruit while the lack of aroma/flavour characterised the firmer samples. Nevertheless,

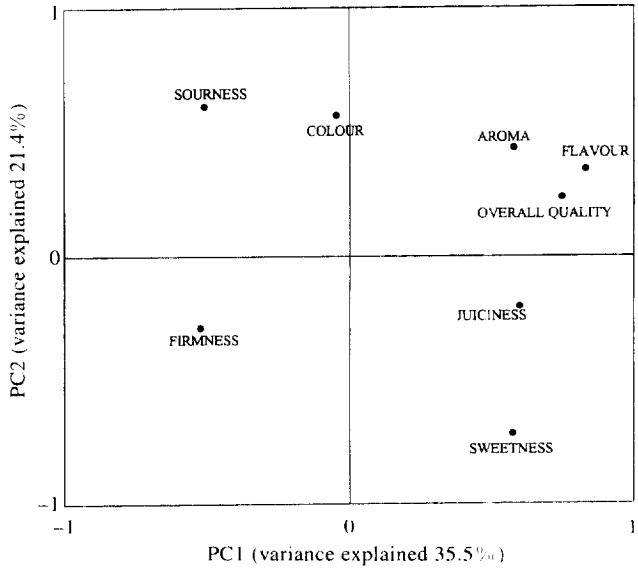

Fig. 4. Correlation plot of the sensory variables on the plane of the first two principal components.

no consistent correlation was found either between firmness and flavour or firmness and overall quality. These data confirm what was previously found by Stec *et al.* (1989) and Mac Rae *et al.* (1989); however, no correlation was found between firmness and sweetness ($R = -0.02$). Overall quality seems to increase in sweeter and juicier kiwifruit samples. Nevertheless, no significant correlation was found between overall quality and the sweetness ($R = 0.26$) and juiciness ($R = 0.25$) ratings.

In Fig. 5, three clusters of kiwifruit are identified by different sensory properties. The PIC 36 genotype (not clustered), seems to have the distinguishing feature of having the best sensory fruit aroma and flavour and overall quality. The genotypes harvested at commercial ripeness are given in the squares (Fig. 5). On comparing Figs 2 and 5, no clear relationships between the results on the chemical data can be found. As regards chemical composition, the genotype samples were classified differently, according to their ripening time, whereas for the sensory data, the clustering does not seem to depend on the time of ripeness. However, in the first case, the genotypes were classified solely on the basis of their soluble sugar and organic acid contents, responsible for the sweet and acid perceptions, but none of the relevant volatile compounds responsible for flavour was taken into consideration. As regards sensory evaluation, aroma and flavour were found to be the best discriminating variables within the data set and were highly correlated with overall quality. In addition, from the correlation matrix of the chemical variables, no significant correlations were found between the perception of sweetness and sourness, nor between the soluble sugars (fructose, glucose and sucrose) and sweetness, nor between the non-volatile acids (malic and citric) and sourness.

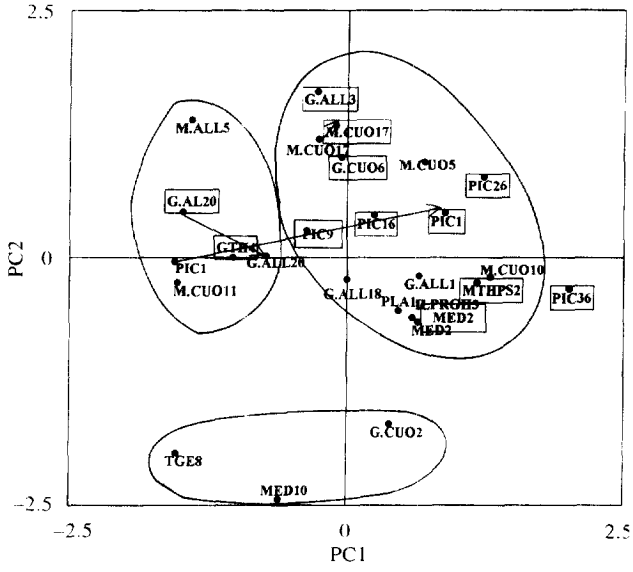

Fig. 5. Score plot of kiwi genotypes on the plane of the first two principal components of a PCA on the sensory variables. Samples are grouped into circles according to Cluster Analysis (CA).

Many other authors have previously failed to demonstrate a clear relationship between the content in chemical compounds and the sensory perception of their intensities in food products, whereas correlations have been demonstrated using solutions of the pure chemical compounds. Kiwifruit is a complex mixture of volatile and non-volatile chemical compounds that contribute to its total flavour and can influence the perception of individual compounds.

CONCLUSION

The multivariate techniques of analysis applied have been useful for classifying the kiwifruit genotypes into two groups: low and high non-volatile acid content. The sensory analysis was useful as it introduced further information other than soluble sugar and non-volatile acid content. Moreover, the overall quality of the kiwifruit genotypes depends more on the aroma of the fruit than on taste (sweet, sour) or mouthfeel.

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