Combined Instrumental and Sensory Evaluation of Flavor of Fresh Bell Peppers (*Capsicum annuum*) Harvested at Three Maturation Stages

Pieternel A. Luning,*,† Ria van der Vuurst de Vries,† Dogan Yuksel,† Truke Ebbenhorst-Seller,† Harry J. Wichers,† and Jacques P. Roozen‡

Agrotechnological Research Institute (ATO-DLO), P.O. Box 17, 6700 AA Wageningen, The Netherlands, and Department of Food Science, Wageningen Agricultural University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

Bell pepper *C. annuum* cv. Mazurka and cv. Evident, at three maturation stages, were evaluated sensorily on flavor attributes. Green bell peppers scored mainly on the attributes bitterness, grassy, cucumber, and green bell pepper aroma, whereas the attributes sweetness, sourness, and red bell pepper aroma were distinctive for the red ones. Sugars and organic acids were determined by high-performance liquid chromatography (HPLC), and concentrations of different ions of the acids were calculated from their dissociation equilibria. Principal component analysis (PCA) demonstrated that HPLC data of fructose, glucose, total sugar, and dry matter content were related to the attribute sweetness in the red maturation stage. HPLC concentrations of citric and ascorbic acid, as well as calculated concentrations of undissociated ascorbic and dissociated citric 1 and citric 2, showed close relationships with the attribute sourness. Moreover, pH and HPLC concentrations of malic, oxalic, fumaric, and pyroglutamic acid and calculated contents of dissociated malic 2, pyroglutamic 1, and oxalic 2 appeared to be negatively related with sourness.

Keywords: *Capsicum annuum; bell pepper; sensory evaluation; flavor; taste; joint principal component analysis*

INTRODUCTION

The market for Dutch export bell peppers (*Capsicum annuum*) has increased in the last decade (Zachariasse and Abrahamse, 1993). Since consumers have become more critical, attention is shifting toward flavor as an important quality parameter for food products. The overall flavor of fruits and vegetables, as perceived during consumption, is influenced by the composition of volatile and nonvolatile compounds, of which some (i.e., pungent principles) stimulate nonspecific or trigeminal neural responses (Chang and Huang, 1989).

A number of studies have dealt with the relations between instrumental data and sensory attributes of nonvolatile flavor compounds and their contribution to total flavor quality in fruits and vegetables. Paterson et al. (1991) showed that in kiwifruits the fructose/glucose ratio was one of the important parameters in the prediction of sweetness of soft ripe fruits, whereas high sucrose contents correlated with unripeness. Furthermore, they observed that the sensory attribute tangy/acid was characteristic for the firm unripe kiwifruits, and that high attribute scores correlated with high citrate concentrations. Watada and Aulenbach (1979) suggested that for tomatoes sourness correlated with citric acid content. Dever et al. (1992) reported that fruitiness and sweetness of apple juice increased while sourness decreased with maturation of apples. Principal component analysis of their instrumental data suggested that, among others, malic acid and titratable acid were negatively related with glucose, fructose, soluble solids, and pH on the first component, representing changes during ripening. However, Watada et al. (1981) indicated that variation of sourness of several apple cultivars could only partly be attributed to titratable acids, soluble solids, and some volatile compounds. Shamaila et al. (1992) observed significant relationships between glucose and fructose concentrations and the sweetness of strawberry samples, but not for pH or titratable acidity. Whereas the concept of sweetness intensity and concentration of sugars has rather been well established (de Graaf, 1988), the relationship between sourness and organic acids has not yet been fully understood (Amerine et al., 1965; Ganzevles, 1987).

In this study, bell peppers were evaluated at three maturation stages by a sensory descriptive panel on flavor attributes perceived while eating. The composition of sugars and organic acids of the different samples was analyzed by high-performance liquid chromatography, and the dissociation of organic acids was calculated at the pH of the samples. These parameters were used to study the relations between sugar concentrations and sweet perception, as well as contents of organic acids and perception of sourness by using joint principal component analysis and partial least squares regression analyses.

MATERIALS AND METHODS

Bell Pepper Samples. Fruits of two commercial Dutch cultivars, i.e., *C. annuum* cv. Mazurka and cv. Evident, were grown and harvested as previously described by Luning et al. (1994a). Three ripening stages were collected at respectively 6, 8, and 10 weeks after fruit setting; bell peppers with similar color appearance, i.e., green (6 weeks), turning (8 weeks), and red (10 weeks), respectively, were selected for experiments. Approximately 10–12 bell peppers were picked from different plants in early morning, and 5–6 fruits were stored at 13 °C for sensory evaluation. The rest of the bell peppers collected were stored at −24 °C for analysis of sugars and organic acids.

* Author to whom correspondence should be addressed (e-mail IN%"LUNING@ATO.AGRO.NL").

† Agrotechnological Research Institute.

‡ Wageningen Agricultural University.

Dry Matter Analysis. Homogenized bell pepper samples of 1 g were dried for 3 h at 70 °C and subsequently dried at 105 °C for 16 h.

Sensory Analysis. Quantitative descriptive analysis (Stone et al., 1974; Stone and Sidel, 1993) was used to evaluate sensory attributes of bell pepper cv. Mazurka and cv. Evident at three maturation stages.

Training of Panel. Fifteen judges, aged from 25 to 35 years, with ample sensory evaluation experience on other fruits and vegetables, generated a descriptive sensory profile for bell peppers (Stone and Sidel, 1993). An assortment of bell pepper varieties with different flavors were offered to the panel to provide a wide range of sensory notes that might be expected in the samples. Afterward, the same assortment of bell pepper cultivars was used for intensity scaling of the perceived attributes over a period of 3 months. Attributes were then checked on their differentiating merits. Finally, the judges made suggestions and established flavor descriptions to characterize flavor attributes of fresh bell peppers, as perceived during eating. The flavor attributes generated were sweetness, sourness, bitterness, sharpness, grassy, cucumber, floral, and green and red bell pepper aroma.

Evaluation of Bell Peppers. Each bell pepper sample was evaluated twice by the panel, and the total procedure was repeated three times. Five fruits were evaluated per session. The judges indicated the intensity of each attribute by using an unstructured line scale anchored with 0 and 100 at the ends. Data were obtained by using the PSA system (OP&P, Utrecht, The Netherlands). Assessors for sensory evaluations were seated in individual air-conditioned booths.

Sugar Analysis. Extraction of sugars was carried out as described for the enzymatic analysis of sugars by Boehringer (1989) with some modifications.

Extraction Procedure. Samples of 5 g of homogenized frozen bell peppers were blended in 50 mL of Milli Q water with an Ultra Turrax (Ika Labortechnik, T25, Janke & Kunkel). The slurry was treated with 1 mL of potassium hexacyanoferrate (II) trihydrate (106 g of $K_4[Fe(CN)_6]\cdot 3H_2O$/L) and 1 mL of stannous acetate (219.5 g of $Zn(CH_3COO)_2\cdot 2\ H_2O$/L) (Merck), respectively, to remove proteins. Solution volume was made up to 100 mL with Milli Q water and filtered (Schleicher & Schull 595$^1/_2$). Aliquots of 1 mL of filtrate were diluted with 4 mL of CaEDTA (200 mg/L) (Fluka). Sep-Pak C_{18} cartridges were activated with 5 mL of ethanol and 10 mL of Milli Q. Aliqouts of 5 mL of diluted filtrate were pretreated with activated Sep-Pak C_{18}; the first 4 mL was discarded and the last milliliter was used for HPLC. Analysis of sugars was carried out as recently described by Lázaro et al. (1989) with the following modifications.

HPLC Analysis. A Waters HPLC system (Milford, MA) was used, which was equipped with a Waters 510 pump and a Waters U6K injector. Sugars were analyzed on a calcium loaded ion exchange column at 85 °C (Waters Sugar-Pak), in combination with a Sugar-Pak guard column. Samples of 10 μL (four replicates) were injected at a flow rate of 0.5 mL/min, using Milli Q as mobile phase. Components were detected with a differential refractometer (Waters 410).

Ascorbic Acid Analysis. Ascorbic acid was determined, separately from the other organic acids, as previously described by Keijbets and Ebbenhorst-Seller (1990) with some modifications.

Extraction Procedure. Samples of 2.5 g of homogenized frozen bell peppers were mixed with 10 mL of methanol, 10 mL of 9.5% oxalic acid (Merck), and 30 mL of water, using an Ultra Turrax. Water was added to the slurry up to 100 mL, mixed, and filtered (Schleicher & Schull 595$^1/_2$). The total ascorbic acid content was determined by reduction of dehydroascorbic to ascorbic acid with homocysteine. The pH of 10 mL of filtrate was adjusted to pH 5 with 2 M KOH, and subsequently adjusted to pH 7 with 0.05 M Tris buffer. Volume was made up to 25 mL with Milli Q. Aliquots of 1 mL were treated for 15 min in the dark with 1 mL of 0.08% DL-homocysteine solution (Fluka) and filtered (Millex 0.45 μm filter, Millipore).

HPLC Analysis. A radially packed μBonda-Pak C_{18} column (5 μm) and a C_{18} guard column (Waters) were used for the analysis of ascorbic acid. Effluents were monitored at 251 nm with a Waters 484 tunable detector. Samples of 10 μL (four replicates) were injected at a flow rate of 1.5 mL/min using 2.5 g of tetrabutylammonium hydrosulfate (Merck) in 55 mL of methanol/L Milli Q as mobile phase.

Table 1. Dissociation Constants (at 25 °C) of Organic Acids Present in Bell Peppers

organic acid	K_1	K_2	K_3
ascorbic acid[a]	8.0×10^{-5}	1.6×10^{-12}	
citric acid[a]	8.7×10^{-4}	1.8×10^{-5}	4.0×10^{-6}
malic acid[a]	4.1×10^{-4}	9.0×10^{-6}	
oxalic acid[a]	6.5×10^{-2}	6.1×10^{-5}	
fumaric acid[a]	9.3×10^{-4}	3.4×10^{-5}	
pyroglutamic acid[b]	5.8×10^{-4}		
shikimic acid[b]	6.2×10^{-5}		

[a] Data obtained from the literature (Chang, 1981). [b] Dissociation constants determined by titration as described by Chang (1981).

Analysis of Organic Acids. Extraction of organic acids was carried out as described for the enzymatic analysis of citric acid by Boehringer (1989) with the following modifications.

Extraction Procedure. Samples of 5 g of homogenized frozen bell peppers were blended with 50 mL of Milli Q in an Ultra Turrax. The slurry was filtered (Whatman GFC) and 1 mL of filtrate was diluted with 4 mL of Milli Q water. Diluted filtrate (5mL) was pretreated with activated Sep-Pak C_{18} cartridges; the first 4 mL was discarded and the last milliliter was used for HPLC analysis.

HPLC Analysis. Organic acids were analyzed on a 60 m Shodex KC811 ion-exchange column (Waters) at 65 °C and combined with a fast fruit juice guard column (Waters). The same HPLC equipment was described for sugar analysis. Samples of 10 μL (four replicates) were injected at a flow rate of 0.7 mL/min using 0.05 M H_3PO_4 as mobile phase. Column effluents were monitored at 210 nm with a Waters 484 tunable detector.

Identification and Quantification. Organic acids and sugars were identified by comparison of retention times with those of authentic compounds prepared in the same way. Quantification of sugars and organic acids was related to peak areas of corresponding compounds.

Measurement of pH. Five-gram samples of bell peppers were homogenized in a Waring blender, and the slurry was vacuum filtered by using a sintered glass funnel (G3). Subsequently, the pH of the filtrate was measured with a pH meter (Metrohm Herisau, E603, Switzerland).

Calculation of Dissociated Organic Acids. The concentrations of organic acids at their different dissociation states were calculated as described by Chang (1981). The calculations were based upon dissociation constants of the individual organic acids (Table 1), the total concentration of the acid as determined by HPLC, and pH values of the bell pepper filtrates. The dissociation constants of shikimic and pyroglutamic acid were determined by a titration method, whereas the others were available from the literature (Chang, 1981). Calculations of the different dissociation forms of an acid were performed as described by Chang (1981).

Statistical Analyses. Instrumental and sensory data sets were subjected to analysis of variance (ANOVA, Genstat 5) to determine least significant differences (lsd) among cultivars and ripening stages. All analyses were carried out four times, and the entire experiment was repeated three times with newly collected bell peppers.

Multivariate statistical analysis, using principal component analysis (PCA), was applied to study the interrelations between sensory data and the relations between instrumental and sensory data, as described by Piggot and Sharman (1986). PCA involves transformation of the original set of p variables ($X_1, X_2, ..., X_p$) from n observations into smaller sets of linear combinations that account for the maximum possible proportion of variance in the original data. In addition, combined data sets were subjected to partial least-squares regression (PLS) as previously described (Martens and Martens, 1986).

Table 2. Mean Score Ratings[a] of Sensory Attributes of Bell Pepper Cultivars Mazurka (MAZ) and Evident (EVI) Harvested at the Maturation Stages Green, Turning, and Red

sensory attribute	green		turning		red	
	MAZ	EVI	MAZ	EVI	MAZ	EVI
sweet	24.6a	24.0a	38.5b	46.4c	42.6d	52.1e
sour	13.0a	11.2a	25.9b	25.5b	28.6b	30.6b
bitter	25.9a	30.7a	16.4b	12.2b	14.5b	11.9b
sharp	12.7a	17.2b	13.2a	13.0a	12.5a	12.9a
grassy	46.2a	47.1a	24.6b	18.0b	12.4c	10.6c
green bp[b]	43.3a	44.5a	29.7b	23.8b	11.8c	7.8c
floral	10.9a	13.9a	9.0a	11.4a	12.1a	12.4a
cucumber	14.4a	17.6b	7.3c	7.5c	7.5c	5.6c
red bp[b]	6.7a	5.7a	25.8b	29.6b	43.3c	50.0d

[a] Means of three experiments; the mean values for each attribute are significantly ($P < 0.05$) different, for ripeness and/or variety, if followed by a different letter. [b] Abbreviation bp, bell pepper.

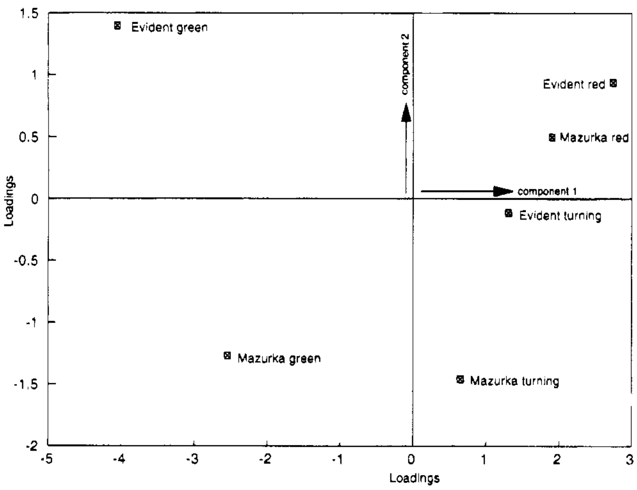

Figure 1. Sample scores of bell pepper cv. Mazurka and cv. Evident in the ripening stages green, turning, and red on the first and second principal component (sensory data set).

RESULTS

Sensory Analyses. Nine flavor attributes were used for sensory profiling of the bell pepper samples (Table 2). Statistical analysis of the mean score ratings of these attributes showed, that scores for sweetness, sourness, and red bell pepper aroma increased significantly ($P < 0.05$) during ripening from green to turning and/or red. On the other hand, scores for bitterness, grassy, green bell pepper, and cucumber aroma decreased significantly ($P < 0.05$). Cultivar differences were observed to a lesser extent. Table 2 shows that cv. Evident had significantly ($P < 0.05$) higher scores for sweetness in the turning and red stages, and for red bell pepper aroma in the red fruits. Green fruits had higher scores for sharp and cucumber aroma in comparison to cv. Mazurka.

Principal component analysis (PCA) was carried out on the mean scores of sensory attributes (Table 2) to study the interrelations of the different ripening stages and cultivars. PCA revealed two principal components explaining 92% of the variance. The sample scores diagram (Figure 1) shows that the maturation stages green, turning, and red for both cultivars were separated along component 1. Figure 2 represents the loadings of the mean score ratings of sensory attributes on the first and second principal component. Obviously, bitterness and "green" flavor attributes such as grassy, green bell pepper, and cucumber aroma have negative loadings on component 1. These attributes are characteristic for the green bell peppers, since these samples also have negative loadings (Figure 1) on the first component. On the positive side of the first component, the red and to a lesser extent the turning fruits have high loadings (Figure 1). Correspondingly, sweet, sour, and red bell pepper aroma have positive loadings on the first component (Figure 2) and therefore are typical for the flavor of ripening bell peppers.

On the second component, green maturation stages of cv. Evident and cv. Mazurka were differentiated; Evident has positive and Mazurka has negative loadings (Figure 1). Figure 2 shows that floral aroma and to a lesser extent sharpness have high positive loadings on the second component and therefore are characteristic for cv. Evident at the green maturation stage.

Instrumental Analyses. Dry matter contents in green, turning, and red fruits were 5.9, 7.3, and 8.4% in cv. Mazurka and 6.1, 7.4, and 9.0% in cv. Evident, respectively. Changes in sugar composition during maturation are shown in Table 3. Glucose and fructose contents increased significantly ($P < 0.05$) from stage green to turning, and subsequently to red, whereas no cultivar differences were observed. However, in the green stage the sucrose concentration of cv. Evident was significantly higher ($P < 0.05$) than in cv. Mazurka. Overall, the highest amounts of sucrose were noticed in the turning stages, whereas a significant ($P < 0.05$) decrease occurred upon further maturation to the red stage (Table 3).

Organic acids of bell peppers were determined by HPLC. Obviously, the composition of organic acids (underlined compounds in Table 4) of both cultivars changed markedly during ripening. From green to turning, and from turning to red ascorbic and citric acid contents increased significantly ($P < 0.05$); likewise malic, fumaric, oxalic, and pyroglutamic acid decreased significantly ($P < 0.05$). Concentrations of shikimic acid dropped significantly from the turning to the red stage. Minor cultivar differences were only observed in the green stage. Malic, fumaric, and oxalic acid contents were significantly ($P < 0.05$) higher and ascorbic acid was significantly ($P < 0.05$) lower for cv. Mazurka compared to cv. Evident.

Organic acids are present in different dissociation states. The degree of dissociation is directly influenced by the pH, therefore, pH was measured to calculate dissociation states in the different maturation stages. Table 4 shows that the pH decreased upon maturation from green to red from 5.7 to 5.1 and 5.6 to 5.0 for cv. Mazurka and cv. Evident, respectively. Ascorbic, pyroglutamic, and shikimic acid are mainly present in the "1" dissociation state. Malic acid exists for a considerable part in the "1" and "2" forms, whereas oxalic and fumaric acid are fully dissociated. All three dissociation forms of citric acid are present in distinct amounts (Table 4). Remarkably, the HPLC concentration of malic acid decreased during ripening, whereas the calculated contents of undissociated malic acid increased significantly ($P < 0.05$). The calculated concentrations of undissociated ascorbic, citric, and shikimic acid also increased significantly ($P < 0.05$) from green to turning and/or red stages (Table 4).

Relationship between Instrumental and Sensory Data. HPLC and sensory data were processed by PCA to study the relationship between sweetness and sugars, and between sourness and organic acids in their different states of dissociation. PCA on the first com-

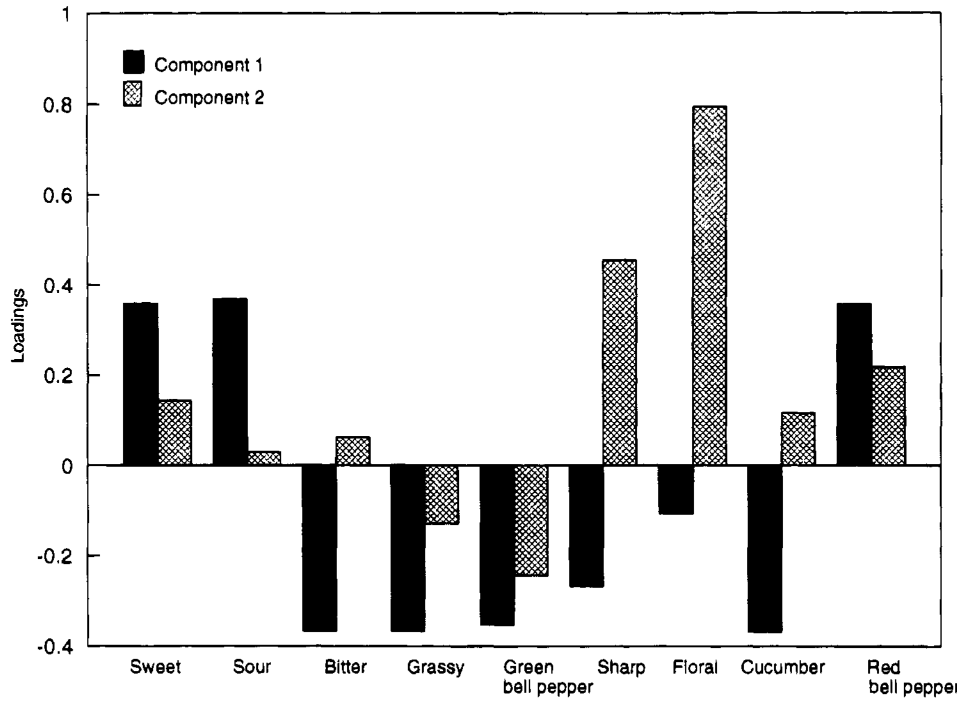

Figure 2. Loadings of mean score ratings of sensory attributes on principal components 1 and 2.

Table 3. Sugar Composition of Bell Pepper Cultivars Mazurka (MAZ) and Evident (EVI) Harvested at the Maturation Stages Green, Turning, and Red

	green		turning		red	
sugar[a]	MAZ	EVI	MAZ	EVI	MAZ	EVI
fructose	1.18	1.06	1.79	1.76	2.58	2.58
glucose	1.38	1.25	1.91	1.87	2.40	2.40
sucrose	0.31	0.80	0.65	0.87	0.19	0.21

[a] Average of three experiments in g/100 g fresh weight; mean coefficient of variation 9.3%.

bined data set (sensory and HPLC data) gave two principal components, which explained 92% of variance. Loadings of sample scores show that bell peppers were differentiated on ripening stage along component 1 (Figure 3). Red maturation stages have high negative loadings, green stages have high positive loadings, and turning bell peppers were in the middle of the first component. Figure 4 shows that sweetness, glucose, fructose, total sugar, and dry matter content have negative loadings on component 1. Sourness, the sum of acids, and ascorbic and citric acid have negative loadings too. Therefore, they are all characteristic for the also negatively loaded turning and red fruits (Figure 3). In addition, the green maturation stages of both cultivars have positive loadings on component 1, while on component 2 cv. Mazurka has negative and cv. Evident has positive loadings (Figure 3). The positive loadings on component 1 of pH, malic, fumaric, pyroglutamic, and oxalic acid (Figure 4) suggest that they are more characteristic for the green fruits as compared to the ripe stages. However, fumaric, pyroglutamic, and oxalic acid also have relatively high negative loadings on component 2, which indicates that they are more characteristic for green fruits of cv. Mazurka than cv. Evident. Figure 4 shows that sucrose and total shikimic acid have positive loadings on component 2, which corresponds with the sample scores of the turning maturation stages of both cultivars, and the green stage of cv. Evident (Figure 3).

Also, PCA was carried out on concentrations of different anions of the acids, calculated from their

Table 4. Composition of Organic Acids, Their Calculated Dissociation States, and pH Values of Bell Pepper cv. Mazurka (MAZ) and cv. Evident (EVI) Harvested at Three Ripening Stages (Average of Three Experiments)[a]

	green		turning		red	
acid	MAZ	EVI	MAZ	EVI	MAZ	EVI
pH	5.7	5.6	5.2	5.2	5.1	5.0
ascorbic[b]	126.1	153.0	167.6	160.9	175.4	177.3
H_2 ascorbic[c]	3.4	5.3	13.6	13.2	20.2	19.1
ascorbic 1[d]	122.7	147.8	153.9	147.8	155.0	158.2
ascorbic 2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
citric	84.0	133.3	261.2	273.2	303.5	350.6
H_3 citric	<0.1	<0.1	0.4	0.4	0.9	0.9
citric 1	3.2	8.0	47.6	50.7	80.7	86.0
citric 2	27.3	51.3	131.8	138.2	154.9	179.9
citric 3	53.5	74.1	81.5	83.9	67.0	83.8
malic	87.7	65.7	31.7	40.0	28.1	33.4
H_2 malic	0.1	0.1	0.2	0.3	0.3	0.4
malic 1	16.9	15.1	13.2	17.0	14.1	16.4
malic 2	70.8	50.6	18.5	23.1	14.0	17.1
oxalic	3.6	1.4	0.1	<0.1	<0.1	<0.1
H_2 oxalic	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
oxalic 1	0.1	0.1	<0.1	<0.1	<0.1	<0.1
oxalic 2	3.5	1.3	0.1	<0.1	<0.1	<0.1
fumaric	0.8	0.5	0.1	0.2	0.1	0.1
H_2 fumaric	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
fumaric 1	0.1	0.1	<0.1	<0.1	<0.1	<0.1
fumaric 2	0.7	0.4	0.1	0.1	0.1	0.1
pyroglutamic	3.6	2.8	2.7	2.2	2.4	2.0
H pyroglutamic	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
pyroglutamic 1	3.6	2.8	2.7	2.2	2.4	2.0
shikimic	4.5	4.9	5.4	5.5	3.6	4.5
H shikimic	0.2	0.2	0.5	0.5	0.5	0.6
shikimic 1	4.3	4.7	4.9	5.0	3.2	3.9

[a] Mean coefficient of variation of pH data is 0.7% and of organic acids is 16%. [b] Underlined compounds are concentrations of organic acids determined by HPLC in mg/100 g fresh weight. [c] Calculated concentrations of undissociatd acids. [d] Calculated concentrations of dissociated acids 1, 2, or 3.

dissociation equilibria, and sensory data. Several of the amounts of acids calculated were far below known threshold values for corresponding organic acids i.e., ±0.0025 g/100 mL (Amerine et al., 1965), and therefore not included in the data set. Two principal components

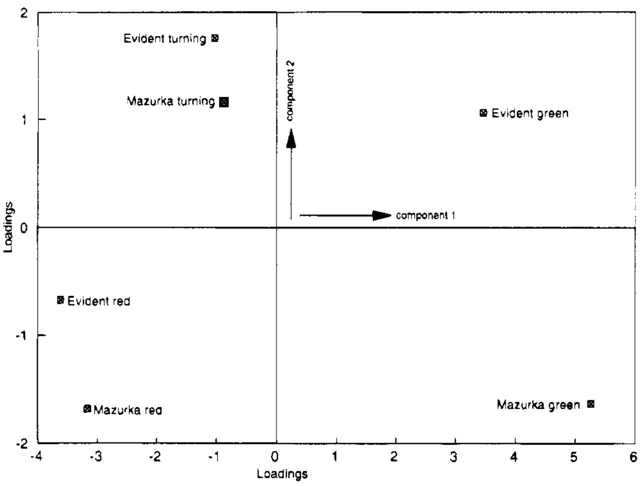

Figure 3. Sample scores of bell pepper cv. Mazurka and cv. Evident in the ripening stages green, turning, and red on the first and second principal component (combined HPLC and sensory data set).

were obtained, which explained 85% of variance of the data set. The loadings diagram of the bell pepper samples was similar to Figure 3. Loadings of calculated instrumental and sensory data are presented in Figure 5. Citric 1, citric 2, undissociated ascorbic acid, and the sum of undissociated and dissociated acids have high negative loadings on component 1, similar to citric acid, ascorbic acid, and sum of acids in Figure 4. However, citric 3 and ascorbic 1 shifted to relatively high positive values on the second component and therefore are more typical for the turning than the red stages (Figure 5). Oxalic 2, pyroglutamic 1, and malic 2 have positive loadings on component 1 and negative loadings on component 2 (Figure 5), which corresponds with loadings of green cv. Mazurka (Figure 3). Malic 1, however, has low loadings on both principal components and is consequently not distinctive for any ripening stage or cultivar.

Partial least-squares regression (PLS) confirmed only principal component 1 in both data sets and showed similar relations for maturation stages as joint PCA. In the first set 75% of variance in the X-data explained 85% of variance in the Y-data, whereas 71% of variance in the X-data explained 86% of variance in the Y-data of the second data set.

DISCUSSION

A complex of factors such as the composition of odor and taste compounds, and their interactions, might influence flavor as perceived during eating. In this study, distinctive sensory flavor attributes of bell peppers, at different maturation stages, were assessed by a trained descriptive panel, and some sensory attributes were related to the intrinsic composition of the samples.

Green maturation stages were characterized by cucumber, grassy, and green bell pepper aroma for both cv. Mazurka and cv. Evident, whereas the ripe stages had a distinct red bell pepper aroma (Table 2; Figures 1 and 2). Likewise, Chitwood et al. (1983) observed that "green" sensory attributes, such as grassy, fresh green bean, and garbanzo bean aroma, discriminated different cultivars of *Capsicum*. They suggested that volatile constituents such as 2-isobutyl-3-methoxypyrazine, 2-*sec*-butyl-3-methoxypyrazine, and (*Z*)-3-hexenol may be responsible for the "green" notes. Luning et al. (1994a) demonstrated by gas chromatography−sniffing port analysis that during ripening of bell peppers mainly volatile compounds with "green" aroma notes disappeared. Moreover, Luning et al. (1994b) suggested relationships between volatile compounds and sensory flavor attributes. Green peppers were apparently char-

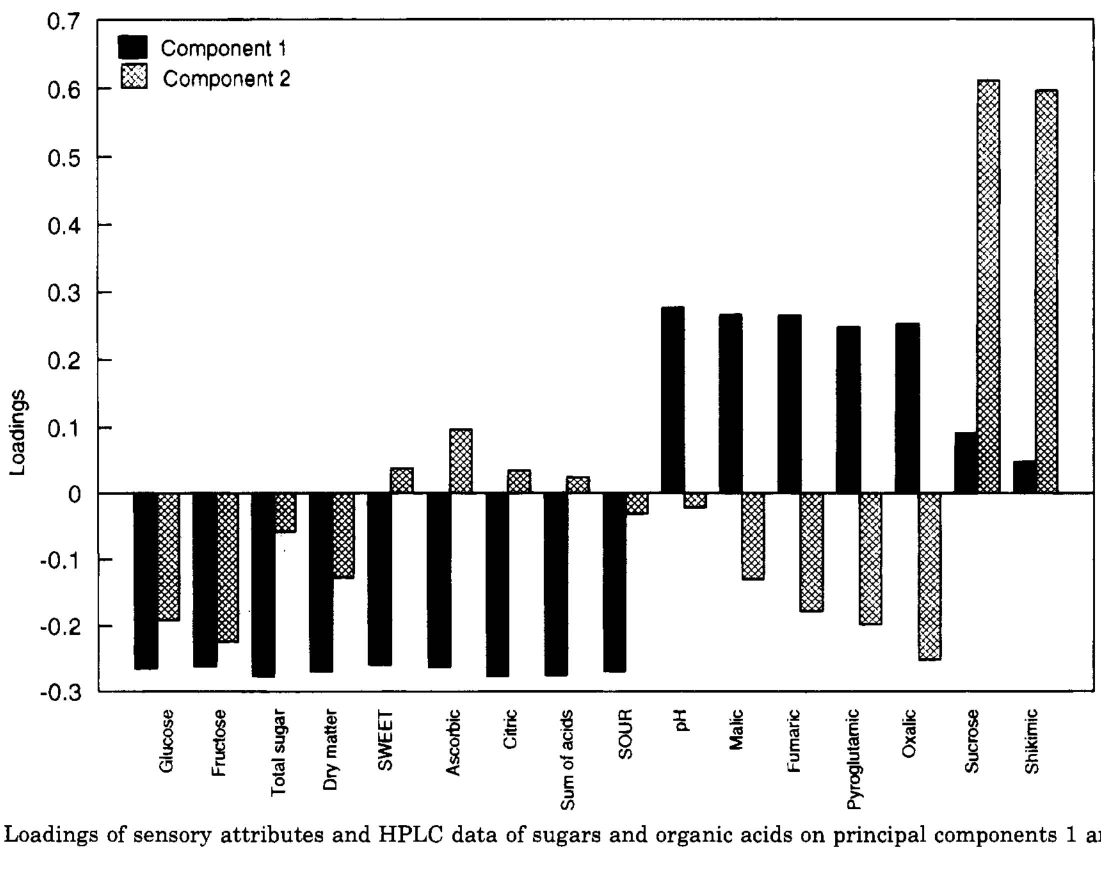

Figure 4. Loadings of sensory attributes and HPLC data of sugars and organic acids on principal components 1 and 2.

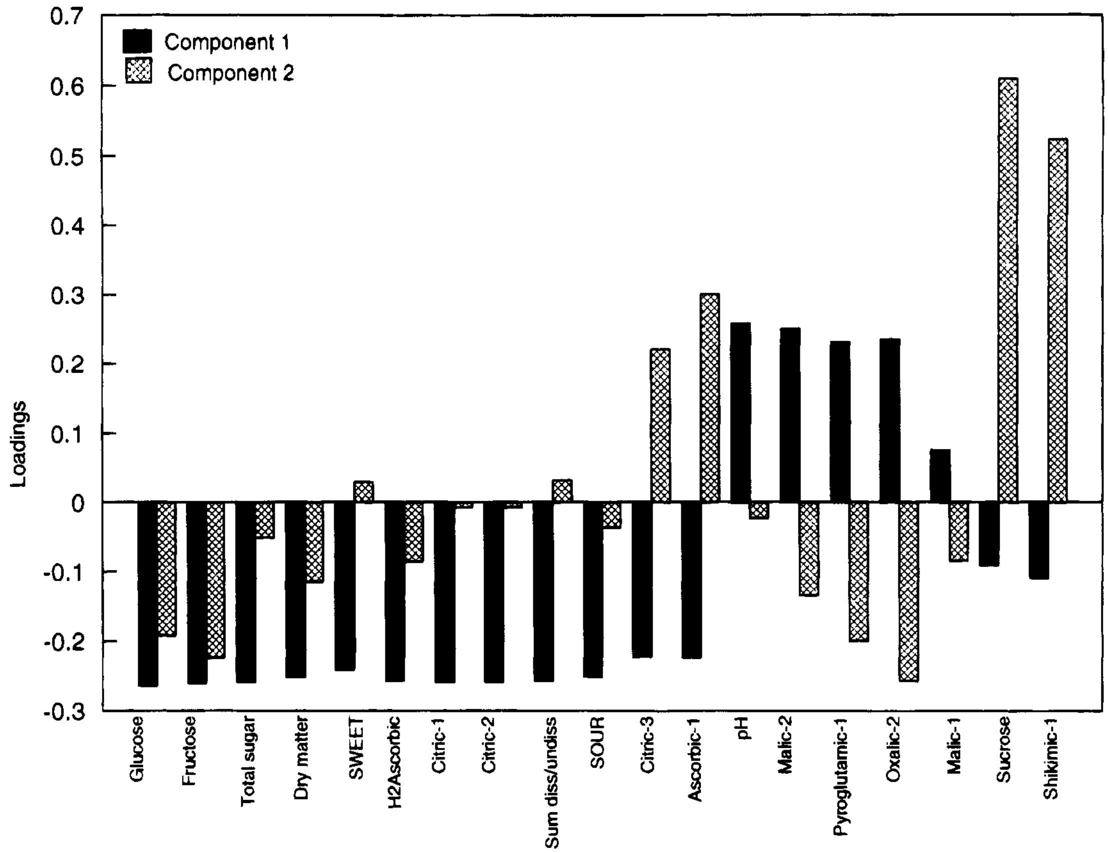

Figure 5. Loadings of sensory attributes, HPLC data of sugars, and calculated amounts of dissociated and undissociated acids on principal components 1 and 2.

acterized by sensory aroma attributes such as, grassy, herbal/grassy, green bell pepper, and fruity/fresh and the presence of high levels of 1-penten-3-one, (*Z*)-3-hexenal, (*Z*)-3-hexenol, 2-isobutyl-3-methoxypyrazine, and linalool, which possess similar flavor notes, indicated that these compounds seem to be responsible for these green related attributes.

Apart from these aroma characteristics, other attributes appeared to be important for the flavor as perceived during eating. Green fruits of cv. Evident were perceived significantly ($P < 0.05$) sharper than green fruits of cv. Mazurka (Table 2). Sharpness of *Capsicum* varieties is caused by capsaicinoids, although sweet peppers in general contain very low amounts (Iwai et al., 1977; Govindarajan, 1985). Bitterness clearly discriminated the green stages from the turning and red ones (Figures 1 and 2). To our knowledge, no specific bitter compounds have been reported in *Capsicum* varieties. Interestingly, some saponins, which can give a bitter perception, were identified in seeds of *C. annuum* fruits (Tschesche and Gutwinski, 1975). Their presence in other parts of the fruit and corresponding sensory characteristics have not been studied.

Sweetness appeared to be typical for ripe stages (Figures 1 and 2) and closely related to glucose, fructose, total sugar, and dry matter content (Figure 4). However, sucrose was not related to changes in sweetness during maturation (Figure 4). Shamaila et al. (1992) observed a significant relation between glucose and fructose levels with the degree of sweetness in strawberry samples, and Dever et al. (1992) suggested that sweetness of apple juice was closely related to glucose, fructose and soluble solids. ANOVA results (Table 2) showed that cv. Evident was sweeter than cv. Mazurka in the turning and ripe stages. These significant differences were, nevertheless, not reflected in differences in sugar composition (Table 3). Probably interactions with other flavor compounds might influence perceived sweetness. Frank et al. (1989) suggested that strawberry flavor induced a significant increase in sweetness of sucrose solutions. Luning et al. (1994b) indicated that the volatile compositions of red fruits of cv. Mazurka and cv. Evident were different; the latter contained higher concentrations of 3-carene, 2-heptanone, 6-methyl-5-hepten-2-one, and dimethyl trisulfide. In addition, Schifferstein and Frijters (1990) demonstrated interactions between sucrose and citric acid on perceived sweet- and sourness. Unfortunately, the concentrations used in their model systems were much higher than in the investigated bell peppers. However, it might be possible, that differences in composition of organic acids and/or other flavor compounds of both cultivars influence the perceived sweetness.

Sourness appeared to be typical for turning and red stages of fresh bell peppers (Figures 1 and 2). The perceived sourness increased significantly ($P < 0.05$) from the green to turning or red maturation stages but not from turning to red stages (Table 2). Citric acid and to a lesser extent ascorbic acid markedly increased during ripening (Table 4). Distinct amounts of citric and ascorbic acid are present in different dissociation states and they probably increased the H^+ concentration. Furthermore, the lowering of pH by 0.5 unit shifted the organic acids in favor of their undissociated states (Table 4). PCA illustrates clearly that total citric and to a lesser extent total ascorbic acid were closely related to sourness, while pH, total malic, fumaric, pyroglutamic, and total oxalic acid suggested a negative relationship (Figure 4). Also, Watada and Aulenbach (1979) and Paterson et al. (1991) suggested correlations between perceived sourness and the citric acid content. PCA on calculated undissociated and dissociated acids revealed that mainly undissociated ascorbic acid and citric acid in the "1" and "2" forms were closely related with sourness, while pH, malic 2, pyroglutamic 1, and oxalic 2 suggested a negative relationship (Figure 5). Ganzevles and Kroeze (1987) studied the perceived sourness of several organic acids in model solutions. They suggested that in contrast to HCl, not only the H^+ concentration but also the undissociated acid appeared to be an important factor in eliciting sourness of organic acids. They implied that HCl sourness and the sourness of organic acids are caused by different receptor processes. According to their theory, undissociated ascorbic acid might be directly important for the sour perception, while the dissociated forms of citric acid influence the sourness by increasing the proton concentration.

LITERATURE CITED

Amerine, A. A.; Pangborn, R. M.; Roessler, E. B. The Sense of Taste. XIII The basic tastes. In *Principles of Sensory Evaluation of Food*; Amerine, M. A., Pangborn, R. M., Roessler, E. B., Eds.; Academic Press: New York, 1965; Chapter 2, pp 75–107.

Boehringer. In *Methods of Biochemical Analysis and Food Analysis. Using Test Combinations*; Boehringer: Mannheim, Germany, 1989; pp 24, 46.

Chang, R. Acids and Bases. In *Physical Chemistry with Applications to Biochemical Systems*; Chang, R., Ed.; MacMillan Publishing: New York, 1981; Chapter 12, pp 310–347.

Chang, S. S.; Huang, A. S. Food Flavors. *Food Technol.* **1989**, *43*, 99–106.

Chitwood, R. L.; Pangborn, R. M.; Jennings, W. GC/MS and Sensory Analysis of Volatiles from Three Cultivars of Capsicum. *Food Chem.* **1983**, *11*, 201–216.

De Graaf, C. Psychophysical Studies of Mixtures of Tastants. Ph.D Dissertation, Agricultural University Wageningen, The Netherlands, 1988.

Dever, M. C.; Cliff, M.; Lau, O. L. Maturity and Ripening Effects on Chemical and Sensory Properties of Apple Juice. *J. Sci. Food Agric.* **1992**, *60*, 355–360.

Frank, R. A.; Ducheny, K.; Mize, S. J. S. Strawberry Odor, but not Red Color, Enhances the Sweetness of Sucrose Solutions. *Chem. Senses* **1989**, *14*, 371–377.

Ganzevles, P. G. J. The Sour Taste. A Filter Paper Study of Adaption and Cross-adaption. Ph.D Dissertation, University of Utrecht, The Netherlands, 1987.

Ganzevles, P. G. J.; Kroeze, J. H. A. The Sour Taste of Acids. The Hydrogen Ion and the Undissociated Acid as Sour Agents. *Chem. Senses* **1987**, *12*, 563–576.

Govindarajan, V. S. Capsicum-Production, Technology, Chemistry, and Quality. Part I. History, Botany, Cultivation and Primary Processing. *CRC Crit. Rev. Food Sci. Nutr.* **1985**, *22*, 109–175.

Iwai, K.; Lee, K.-R.; Kobashi, M.; Suzuki, T. Formation of Pungent Principles in Fruits of Sweet Pepper *Capsicum annuum* L. var. *grossum* During Post-Harvest Ripening under Continuous Light. *Agric. Biol. Chem.* **1977**, *41*, 1873–1879.

Keijbets, M. J. H.; Ebbenhorst-Seller, G. Loss of Vitamin C (L-ascorbic acid) During Long-Term Cold Storage of Dutch Table Potatoes. *Potato Res.* **1990**, *33*, 125–130.

Lázaro, M. J.; Carbonell, E.; Aristoy, M. C.; Safón, J.; Rodrigo, M. Liquid Chromatographic Determination of Acids and Sugars in Homolactic Cucumber Fermentations. *J. Assoc. Off. Anal. Chem.* **1989**, *72*, 52–55.

Luning, P. A.; de Rijk, T.; Wichers, H. J.; Roozen, J. P. Gas Chromatography, Mass Spectrometry and Sniffing Port Analyses of Volatile Compounds of Fresh Bell Peppers (*Capsicum annuum*) at Different Ripening Stages. *J. Agric. Food Chem.* **1994a**, *42*, 977–983.

Luning, P. A.; Yuksel, D.; Roozen, J. P. Sensory Attributes of Bell Peppers (*Capsicum annuum*) Correlated with Composition of Volatile Compounds. In *Trends in Flavour Research*; Maarse, H., van der Heij, D. G., Eds.; Elsevier Science Publishers: Amsterdam, 1994b; pp 241–248.

Martens, M.; Martens, H. Partial Least Squares Regression. In *Statistical Procedures in Food Research*; Piggot, J. R., Sharman, K., Eds.; Elsevier Science Publishers: London, 1986; Chapter 9, pp 293–359.

Paterson, V. J.; MacRae, E. A.; Young, H. Relationships Between Sensory Properties and Chemical Composition of Kiwifruit (*Actinidia deliciosa*). *Sci. Food Agric.* **1991**, *57*, 235–251.

Piggot, J. R.; Sharman, K. Methods to Aid Interpretation of Multidimensional Data. In *Statistical Procedures in Food Research*; Piggot, J. R., Sharman, K., Eds.; Elsevier Science Publishers: London, 1986; Chapter 6, pp 181–232.

Schifferstein, H. N. J.; Frijters, J. E. R. Sensory Integration in Citric Acid/Sucrose Mixtures. *Chem. Senses* **1990**, *15*, 87–109.

Shamaila, M.; Powrie, W. D.; Skura, B. J. Sensory Evaluation of Strawberry Fruit Stored at Modified Atmosphere Packaging (MAP) by Quantitative Descriptive Analysis. *J. Food Sci.* **1992**, *57*, 1168–1172.

Stone, H.; Sidel, J. Descriptive Analysis. In *Sensory Evaluation Practices*. Stone, H., Sidel, J. L., Eds.; Academic Press: San Diego, CA, 1993; Chapter 6.

Stone, H.; Sidel, J. L.; Joel, O. S.; Woolsey, A.; Singleton, R. C. Sensory Evaluation by Quantitative Descriptive Analysis. *Food Technol.* **1974**, *28*, 24–34.

Tschesche, R.; Gutwinski, H. Capsicosid, a Bisdesmosidic 22-Hydroxy-Furostanol-Glycosid from Seeds of *Capsicum annuum* L. *Chem. Ber.* **1975**, *108*, 265–267.

Watada, A. E.; Aulenbach, B. B. Chemical and Sensory Qualities of Fresh Market Tomatoes. *J. Food Sci.* **1979**, *44*, 1013–1016.

Watada, A. E.; Abbott, J. A.; Hardenburg, R. E.; Lusby, W. Relations of Apple Sensory Attributes to Headspace Volatiles, Soluble Solids, and Titratable Acids. *J. Am. Soc. Hortic. Sci.* **1981**, *106*, 130–132.

Zachariasse, L. C.; Abrahamse, A. P. *Tuinbouwcijfers 1993*; Centraal Bureau Statistiek: Voorburg, The Netherlands; 1993; Vol. 1, Chapter 9, p 105.

Registry No. Supplied by the Author: Sucrose, 57-50-1; fructose, 57-48-7; glucose, 50-99-7; L-ascorbic acid, 50-81-7; citric acid, 77-92-9; L-malic acid, 97-67-6; oxalic acid, 144-62-7; fumaric acid, 110-17-8; shikimic acid, 38-59-0; L-pyroglutamic acid, 98-79-3.

Received for review May 19, 1994. Revised manuscript received September 7, 1994. Accepted September 20, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, November 1, 1994.