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SCIENCE DIRECT®
Food Quality and Preference 17 (2006) 376–386
Food Quality and Preference
www.elsevier.com/locate/foodqual

Perception of flavour in standardised fruit pulps with additions of acids or sugars

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Received 11 November 2004; accepted 30 April 2005
Available online 16 June 2005

Abstract

We hypothesised that adding sugars or acids to pulps derived from fruit of different genetic background, or of lower or higher carbohydrate status should give similar results until a threshold for sweetness or acidity perception in the pulp background was reached. Pulps made from fruit of *Actinidia deliciosa* 'Hayward'—a fresh sweet-acid kiwifruit—and *A. chinensis* 'Hort16A'—a sweet tropical flavoured kiwifruit, were compared. Flavour volatiles and background sugar and acid composition differed between the two fruit. Using high (14.1–14.5%) or low (11.5–11.9%) soluble solids (SS) pulps, trained tasters perceived added sugar as sweeter, confirming earlier research with consumers. Added acids (7 mmoles H^+ 100 g^{-1}) reduced perceptions of sweetness and tasters perceived quinic acid to have a strong impact on the pulps tasted, resulting in a greater perception of acidity than addition of citric, ascorbic or malic acids. However perceptions of acidity also depended on background pulp composition. Addition of sugar or acid to pulps affected measured headspace volatiles. Volatile compounds affected included mid-chain length aldehydes, alcohols and esters, but only a few showed significant alteration. For example, hexanal, (*E*)-3-hexen-1-ol and (*Z*)-3-hexenol increased significantly when malic acid was added to 'Hayward' pulps. We suggest that the release of alcohols was associated with change in acidity and pulp metabolism rather than a 'salting out' effect. Perceived changes to banana and lemon flavours with both sugar and acid addition appear to be due to interactions in taster mouths or associated memories rather than release of specific volatiles.

Keywords: Kiwifruit pulp; Flavour volatiles; Sugar; Acid perception; *Actinidia*

1. Introduction

Consumer preference of fruit is strongly influenced by sweetness, acidity and characteristic flavours. For example, the commercial green kiwifruit (*Actinidia deliciosa* (A. Chev.)) C.F. Liang et A.R. Ferguson var. *deliciosa* ('Hayward') is described by consumers as having fresh, sweet-acid flavours, while the commercial kiwifruit sold as ZESPRI^{TM} GOLD (A. *chinensis* Planch. var. *chinensis* 'Hort16A') is described as having sweet and fruity flavours. Flavour was identified as a key differentiator in a consumer preference mapping study on kiwifruit where consumers segregated into at least two groupings based on preference for either the gold or green fruit (Jaeger, Rossiter, Wismer, & Harker, 2002). Sweetness and acidity are important aspects of fruit taste. Sugar and acid content can differ markedly within families of kiwifruit, peach or strawberry (Cheng et al., 2004; Monet, Guye, Roy, & Dachary, 1996; Shaw, 1990), and wide variation occurs in sweet or acid perception of such fruits (Wismer et al., submitted for publication). For example, in comparing apples with genetically related backgrounds (Harker et al., 2002), trained tasters with pre-screened sensitivity to acid and sugar could detect a difference in 0.08% titratable acidity (TA) between

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0950-3293/$ - see front matter
doi:10.1016/j.foodqual.2005.04.011

different apples. Although olfactory-GC (Jordan, Margaria, Shaw, & Goodner, 2002) and sensory methods which combine chemical analysis of fruit in association with taste panels can offer useful insight into consumer responses (Jaeger et al., 2002), fresh fruit are living organs and therefore inherently variable and responsive to environment. This can confound attempts to understand human responses to fresh fruit, and to determine the relative impact of different flavour components.

To simplify the study of perception and impact of different flavour components, model solutions have most commonly been used. For example, sugar addition has been shown to suppress sourness, and acid to suppress sweetness in solution studies (Bonnans & Noble, 1993). Acid addition varying in both type and concentration can affect not only sourness but also bitterness and astringency (Hartwig & McDaniel, 1995; Rubico & McDaniel, 1992). Sugar has also been shown to affect release of volatile components in model solutions (Friel, Linforth, & Taylor, 2000). In particular, more hydrophilic compounds were retained and more hydrophobic compounds released as sucrose concentrations increased. Research using aqueous solutions modelled on kiwifruit demonstrated that some volatiles are important in consumer liking of kiwifruit (Ball, Murray, Young, & Gilbert, 1998; Gilbert, Young, Ball, & Murray, 1996; Young, Stec, Paterson, McMath, & Ball, 1995). Three volatile compounds affected perception of sweetness (methyl benzoate, ethyl butanoate and hexanal), while (*E*)-2-hexenal, hexanal and ethyl butanoate increased perception of kiwifruit flavour. In particular, ethyl butanoate impacted on flavour acceptability of a kiwifruit-like sugar-acid solution and was recognised as a component of the 'characteristic kiwifruit flavour'. Aqueous solutions, however, do not allow for the more complex sensory interactions that take place in the presence of real fruit tissue. Differences in flavour perception can occur because of altered volatile release and detection during eating as opposed to drinking (Normand, Avison, & Parker, 2004).

In order to test hypotheses relating to key flavour components of kiwifruit in a more realistic background, Rossiter, Young, Walker, Miller, and Dawson (2000) presented a low volatile, low dry matter 'Hayward' kiwifruit pulp (to provide a low starting sugar concentration and minimal interference from flavour volatiles) to consumer panels, and found that flavour acceptability increased with increasing sucrose addition in the range 11–14%SS. Using the same experimental system and trained panels, added sugar up to 20%SS resulted in increased sweetness, decreased acidity and increased banana flavour (Marsh, Rossiter, Lau, Walker, & MacRae, 2003). Similarly, additions of 7 mM concentrations of each acid led to a reduction in sweetness, banana flavour and 'characteristic kiwifruit' flavour. The type of sugar added had no effect on either sweetness or acidity, but the type of acid appeared to matter. There are large variations in composition of the three major fruit acids (citric, malic and quinic) in kiwifruit germplasm (Cheng et al., 2004) and within an individual fruit (MacRae, Lallu, Searle, & Bowen, 1989). For example, ascorbic acid varies between 15 and 2000 mg 100 g FW^{-1} within the kiwifruit germplasm (Ferguson & MacRae, 1991) and added ascorbate in this range reduced sweetness and increased acidity, lemon flavour and astringent aftertaste (Marsh et al., 2003). Similar experiments have been carried out using pulps made from ripe store-purchased mango (Malundo, Shewfelt, Ware, & Baldwin, 2001) showing that citric acid or sugar addition affected sweet, peachy, pine/turpentine, astringent and biting flavours. Only citric acid affected perceptions of sourness.

Pulps therefore provide a robust option for assessing potential new fruit flavours in *Actinidia*, and to test the role of key impact compounds. We hypothesised that additions of sugars and acids to high DM 'Hayward' pulps and to 'Hort16A' based pulps would result in modified perceptions of sweetness or acidity in a manner similar to previous experiments with low DM 'Hayward' until a taste threshold is met. Quinic acid is a major fruit acid in *Actinidia* and we explored the effect it has on taste perception in a fruit background in more detail. Finally the reported experiments in 'Hayward' pulps and mango pulps indicated that some flavours (e.g. banana) were altered with addition of sugar or acid. Hence, we also examined volatile headspace concentrations after addition of sugars or acids, and assessed whether any alteration compared to the controls might lead to changed taster perception.

2. Experimental

2.1. Low and high DM pulps

Fruit of *A. deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa* 'Hayward' or *A. chinensis* Planch. var. *chinensis* 'Hort16A' were harvested at commercial maturity and stored at 0 °C. Following 12 weeks storage, fruit were ripened at 20 °C until required for panels. Ripe fruit were pre-screened for SS with a refractometer (Atago) and pH with a pH meter (Shindingen KS701). Fruit above 14%SS or below 13.5%SS were segregated to make high (14.1% 'Hort16A'; 14.5% 'Hayward') or low (11.4% 'Hayward'; 11.9% 'Hort16A') SS pulps. Pulps were prepared according to Rossiter et al. (2000), and included core and seeds to form a uniform slightly chunky (<5 mm) mixture. On each day of testing, fruit were presorted, peeled, pulped (2 kg) and weighed into 300 g aliquots in separate glass beakers. Filtered water was added to control pulps in volumes matching the added sugars or acids.

2.2. Chemical analysis

2.2.1. Sugar and acid analysis

Starter pulps were analysed for sugar and acid content after extraction according to Richardson, Marsh, and MacRae (1997) (Table 1).

2.2.2. Volatile analysis

Approximately 1 g of the pulped kiwifruit was weighed into a 50 ml test-tube, and the fruit pulp was stirred using a magnetic stirrer. The vessel was covered with a glass lid fitted with a sealed ground glass joint inlet socket containing a gas line and a volatile sorbent cartridge (100 mg Chromosorb 105). The headspace in the test-tube was purged with dried purified air at 25.0 ml min^{-1} whilst being trapped for 15 min at 21 °C. The sorbent cartridge was then placed inside a glass tube and stored at −20 °C. Immediately prior to analysis by gas chromatography (GC) with simultaneous flame ionisation (FID) and mass spectrometric detection (MS), the cartridges were allowed to warm to room temperature and were dried with a N_2 gas flow at 12 ml min^{-1}, 35 °C for 15 min. Headspace volatiles were measured in duplicate for the 'Hort16A' with acid addition and 'Hayward' with sugar addition, in triplicate for 'Hayward' with acid addition and as a single measurement for 'Hort16A' with sugar addition. The data has been reported with ethanol excluded as this compound was always present and dominated analysis.

2.2.3. GC/MS

Methods were as in Young, Rossiter, Wang, and Miller (1999) except that desorption took place at 150 °C, and the GC oven programme was altered from 30 °C per min until 102 °C, followed by 5 °C per min until 210 °C, which was maintained for 5 min. The mass spectrometer operated in electron impact ionisation mode at 70 eV with a scan range 30–320 amu. Component identification was assisted with mass spectra of authentic standards, library spectra (NIST 98, Wiley 7 and in-house) and GC retention indices. Semi-quantitative data was obtained from the FID. This involved an injection of a pentane standard containing known concentrations of chemicals representative of most classes of volatile compounds identified in kiwifruit (e.g. ethyl butanoate to represent esters etc.). An average response factor was then calculated for the chemicals in the standard from the areas and this was applied to the peak areas from the samples to obtain semi-quantitative concentrations.

2.3. Experimental design

2.3.1. Sugar addition

Stock sugar solutions were made to correspond to the ratio of sugars commonly measured in 'Hayward' and 'Hort16A' (Marsh et al., 2003). The combined sugar solution was then added to each high or low SS pulp in two increments of 2% sugar, with matching volumes of filtered water added to the controls. Measured SS and DM of the final test pulps is presented in Table 2.

2.3.2. Acid addition

Stock solutions of malic, citric, ascorbic and quinic acids were made representing 3 moles per litre in H^+ ion (Marsh et al., 2003). The individual acids were added to low DM 'Hort16A' and 'Hayward' pulps at 7 mmoles H^+ 100 ml^{-1}, equivalent to half the range of natural variation found within 'Hayward'.

2.4. Sensory panels

2.4.1. Trained panels

Eight panellists were selected from a pool of 32 trained panellists, who had been previously screened for their sensitivity to fruit acidity. These panellists were subjected to training over a period of eight weeks. Over the course of the training, definitions of the odour, flavour and textural attributes found were generated and appropriate reference standards were established. All attributes were assessed using 150 mm unstructured line scales anchored at 0 = absent and 150 = extreme. Sucrose at 30 g l^{-1} and 60 g l^{-1} were used respectively for low (35) and high (120) sweetness. Malic acid at 1.0 and 1.5 g l^{-1} represented low (30) and high (100) acidity. Undiluted 'Ocean Spray' Cranberry juice represented high astringency (150). Commercial 'Hansells' lemon essence (100 μl per litre) and 'Quest' banana essence (200 μl per litre) represented lemon (40) and banana (120) flavours, and 30 μl per litre cis-3-hexen-1-ol represented

Table 1
Mean concentrations of sugars and acids in the control pulps before addition of test solutions

Pulp	Organic acid (mg g^{-1} FW)			Sugar (mg g^{-1} FW)			
	Malate	Citrate	Quinate	Inositol	Sucrose	Glucose	Fructose
Low DM 'Hayward'	2.2	8.7	12.6	1.9	8.2	30.0	40.7
High DM 'Hayward'	4.3	12.4	20.9	1.9	11.5	35.9	46.2
Low DM 'Hort16A'	3.3	15.2	9.7	1.4	9.6	30.4	40.5
High DM 'Hort16A'	5.5	11.2	20.6	1.3	15.0	39.3	49.6

Table 2
Volatile compounds in the base pulps prior to addition of sugars or acids

	'Hayward'				'Hort16A'		
	Acid control	High DM	Low DM		Acid control	High DM	Low DM
(*E*)-2-Hexenal	1666.5	5533.4	5244.7	Ethyl butanoate	2446.8	219.5	21018.3
Ethyl butanoate	813.3	77.2	60.1	Ethyl acetate	71.8	12.2	314.5
Methyl butanoate	480.0	13.0	26.2	Methyl butanoate	63.5	8.1	430.5
Ethyl acetate	207.4	200.0	61.9	(*E*)-2-Hexenal	32.9	20.4	45.3
2-Hexen-1-ol	203.5	269.5	498.3	1-Butanol	26.8	10.0	245.1
1-Hexanol	110.0	132.5	208.2	Ethyl propanoate	18.7		
Ethyl propanoate	46.9			Ethyl hexanoate	13.9		53.7
Hexanal	46.7	383.2	214.2	Hexanal	13.6	13.8	8.5
1-Penten-3-ol	28.1	32.0	45.2	Nonanal	13.0	20.4	
Methyl acetate	19.4	16.0	52.7	Decanal	12.8	4.6	0.8
β-Pinene	14.4	20.9	47.5	(*E*)-2-Hexen-1-ol	9.8		
1-Pentanol	12.9	7.7	21.7	Octanal	9.6	1.4	7.6
Ethyl 2-methylpropanoate	12.9	9.3	8.2	Ethyl pentanoate	5.3		
Ethyl hexanoate	11.3			Ethyl 2-methylpropanoate	4.2		
(*E*)-3-Hexen-1-ol	10.6	5.8	11.0	Unknown	4.2		
(*Z*)-3-Hexen-1-ol	9.9	3.0	30.6	1-Hexanol	3.3		
Methyl hexanoate	9.8			1,8-Cineole	3.1	30.1	0.7
α-Pinene	9.3	11.9	26.0	β-Pinene	2.9		10.5
(*Z*)-3-Hexenal	7.3	16.9	20.7	Unknown	1.9		
Octanal	5.6	6.3	19.7	Unknown	1.0		
Methyl 2-methylpropanoate	5.5	5.7	4.8	Ethyl benzaldehyde	0.7		
Benzaldehyde	2.8	9.6	4.6	Unknown	0.7		
Acetone		15.8	14.7	3-Methylbutyl acetate		0.1	
Decanal		24.7	54.7	Acetic acid		0.5	6.9
Ethyl propanoate		81.9	30.7	Benzaldehyde		3.4	3.4
Heptanal		5.6	9.6	Dimethyl benzaldehyde		12.8	
Nonanal		6.6	34.3	Dimethyl sulfoxide		0.1	
Tetrahydrofuran		36.9	26.8				
(*Z*)-2-Hexenal		59.0	63.4				
Total	3734.2	6984.3	6840.7	Total	2760.6	357.3	22145.8

Compounds are given as ng g^{-1} pulp. The concentration of some volatiles are different in the low and high DM pulp in particular due to differences in fruit metabolism during growth and storage.

'grassy' (120). Another commercial extract, 'GR Gooseberry flavour' was used to anchor trained panellists at 100 for characteristic 'Hayward' flavour, and 'Sparkling Ribena' was used to anchor trained panellists at 100 for typical 'Hort16A flavour'. Testing took place over a one-week period. For each trial, samples were presented monadically according to randomised complete block designs with a maximum of six samples assessed each day. Ten-minute breaks were enforced after the first three samples to reduce panellist fatigue. Testing was carried out in sensory booths, which were maintained at 20 °C with a positive airflow to remove odours from the testing area. Microlene™ filtered water and plain water crackers were provided as palate cleansers.

2.4.2. Consumer panels

We conducted a consumer study to confirm that differences found by trained panellists reflected consumer responses to altered acidity. Over two days, consumers ($n = 42$) with a range of ages and ethnic backgrounds were presented with four different acid samples (malic acid, citric acid, quinic acid, potassium citrate/citric acid) at 7 mmoles H^+ 100 ml^{-1} addition to a Low DM 'Hayward' pulp. Water controls were included, and consumers were asked to rank the samples for acidity and to place them on a labelled magnitude scale (150 mm). Testing was carried out in booths as above.

2.5. Data analysis

In each case data were subjected to a repeated measures analysis of variance (ANOVA) to determine if differences between treatments were significant. Tukey's least significant differences (LSDs) were calculated to compare differences between means (following significance in the ANOVA). All effects and interactions were evaluated at the 5% level unless otherwise stated.

Linear discriminant analysis (LDA) was carried out on the samples with replicated data. LDA is a multidimensional scaling method which is used to find product positions in a space where the ratio of the between products sum of squares to the within products sum of squares has been maximised. The chemicals were ordered in terms of decreasing coefficients of variation. Those with high coefficients would be expected to account for most of the information in the map. As the LDA was run on log concentrations, where a chemical component was not detected by chemical analysis in a single replicate, the minimum value detected for that component in other replicates was multiplied by 0.5 to prevent zero values in the data. Each ellipse represents a 95% confidence region of the mean of the replicates. Chemicals that made a major contribution to the discriminant function were determined and reported in the text. Data that had not undergone a log transformation was also analysed and compared. Analysis was performed using the LDA function found in MASS library for S Plus 6.1 for Windows contributed by Venables and Ripley (1997).

3. Results

3.1. Kiwifruit pulps

'Hayward' pulps were generally more acid, astringent and grassy than 'Hort16A' pulps (Tables 2 and 3). Both had characteristic flavours. The high DM 'Hayward' pulp was perceived to be more acidic and lemon flavoured than the low DM 'Hayward' pulp, but 'Hort16A' pulps were similar to one another.

There were differences in volatile content between the high and low DM pulps of each genotype. For 'Hort16A', low DM pulps had significantly ($p < 0.05$) greater volatiles (22,146 ng g^{-1} fresh weight (FW) versus 357 ng g^{-1} FW), reflected in significantly elevated concentrations of ethyl acetate, methyl butanoate, 1-butanol and (*E*)-2-hexenal (Fig. 1a) and ethyl hexanoate. By contrast 'Hayward' low DM pulps showed significantly higher 1-pentanol, and methyl acetate ($p < 0.05$) than high DM pulps (Fig. 1b). Although total volatiles in the pulp headspace were marginally higher in the high DM pulps (6984 ng g^{-1} FW for the average of high DM versus 6841 ng g^{-1} FW for the average of low DM) this difference was not significant. There were a number of compounds present in pulps of 'Hort16A' and not found in 'Hayward' (e.g. 1,8 cineole, dimethyl sulfoxide (DMSO), 1-butanol) and vice versa (e.g. several mid-chain alcohols and aldehydes and methyl esters) (Table 2).

3.2. Increasing SS through sugar addition

When sugar solutions were added to high and low DM fruit pulps resulting in an increase of 2%SS or 4%SS (Table 3), the ratings for sweetness were directly related to SS, regardless of the manner (pre-sorting fruit or sugar addition) in which the increase in SS was achieved. As had been found previously, a SS lower than 15 % was distinctly perceived as less sweet than a SS of 16–17%, and pulps with a very high SS (>18%) were perceived as most sweet. Results were the same whether a 'Hayward' or 'Hort16A' background pulp was used, and sweetness ratings were similar if %SS of the pulp was similar.

Table 3
Mean sensory score for attributes recorded by trained panellists (150 mm line scale) in pulp with various sugar additions for (A) low dry matter and high dry matter 'Hayward' pulp, and (B) low and high dry matter 'Hort16A' pulp ($n = 8$; averaged over 2 days 'Hayward' and 1 day 'Hort16A' and tested for day effects)

	Low DM control	Low DM + 2%	Low DM + 4%	High DM control	High DM + 2%	High DM + 4%	$LSD_{.05}$
(A) 'Hayward'[a]							
Soluble solids (measured)[b]	*11.4*	*13.3*	*15.4*	*14. 5*	*16.3*	*18.25*	
Dry matter (measured)[b]	*13.75*	*15.0*	*16.75*	*16.0*	*17.3*	*19.35*	
Sweetness	63.4d	84.7c	101.4ab	93.6bc	102.7ab	116.3a	15.4
Acidity	83.6c	80.9c	85.2bc	98.2ab	104.6a	89.4bc	13.3
Lemon flavour	59.7b	65.7ab	64.0ab	74.1a	68.2ab	66.2ab	13.4
Banana flavour	28.7b	39ab	47.6a	34.2ab	41.2ab	44.3a	15.4
Characteristic 'Hayward' flavour	54.0b	60.9ab	62.0ab	58.9ab	67.2a	58.9ab	10.8
Astringency	70.9ab	59.3bc	63.8abc	73.2a	72.2a	57.1c	12.3
(B) 'Hort16A'[a]							
Soluble solids (measured)[b]	*11.9*	*14.0*	*15.6*	*14.1*	*16.1*	*18.3*	
Dry matter (measured)[b]	*12.7*	*14.7*	*16.5*	*16.0*	*17.6*	*19.0*	
Sweetness	73.6d	94.1bc	106.6abc	88.5cd	107.5ab	115.8a	23.1
Acidity	64.7a	60.2a	62.5a	76.2a	64.7a	66.7a	15.0
Lemon flavour	62.4a	53.4a	54.3a	59.8a	58.8a	55.5a	22.0 ns
Banana flavour	40.5ab	56.1a	59.2a	30.0b	28.3b	48.1ab	23.5
Characteristic 'Hort16A' flavour	57.5b	58.9ab	75.5ab	72.7ab	87.5ab	88.8a	31.2
Astringency	52.4a	44.1a	40.1a	47.1a	52.8a	50.8a	16.1 ns

[a] Numbers with the same letter following are not different at $p < 0.05$.
[b] Mean soluble solids and dry matter values were measured in the individual bulk pulp samples after sugar addition.

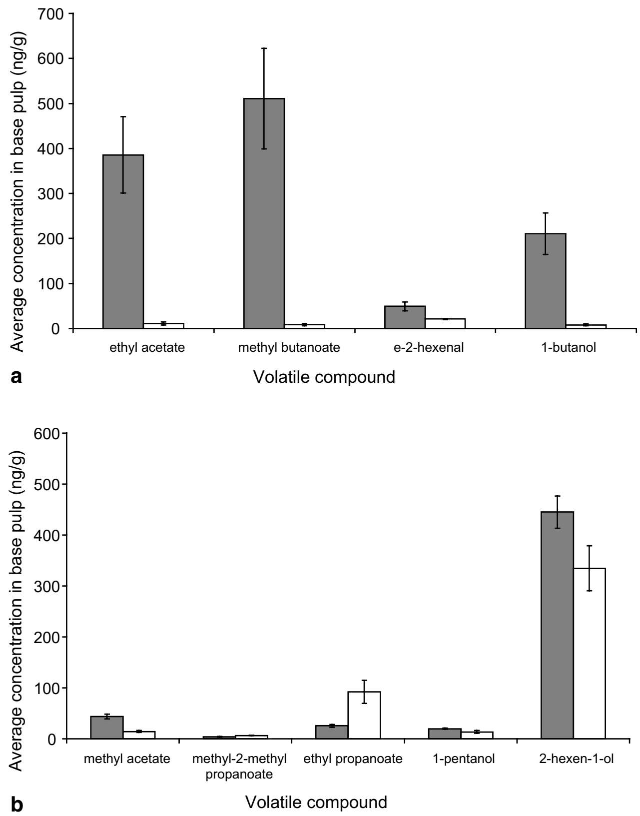


Fig. 1. Concentrations of key volatiles that differed significantly between low and high DM pulps of (a) ‘Hort16A’ pulps and (b) ‘Hayward’ pulps. Data are means of all low DM (grey) pulps compared to all high DM (white) pulps. The bars represent the standard error of three replicate measurements.

Addition of sugar did not remove differences in perception of acidity between the ‘Hayward’ low and high DM pulps and did not alter the acidity rating for ‘Hort16A’ pulps (Table 3). Astringency (Table 3) was rated the same in high and low DM control pulps for both ‘Hayward’ and ‘Hort16A’ and the only effect of adding sugar (4%) was to decrease the perceived astringency in the ‘Hayward’ high DM base pulp.

By contrast, banana flavour rating increased with the addition of sugar to ‘Hayward’ pulps and was not related to the initial SS of the pulp base (Table 3). Both of the ‘Hort16A’ pulps showed a slight, non significant, increase in rating of banana flavour with addition of sugar, but banana flavour bore no relationship to SS. For example, 14%SS from sugar addition to a low DM pulp had a significantly higher banana rating than a high DM base pulp of 14.1%SS. Characteristic flavours did not alter significantly with the addition of sugar (Table 3).

‘Hort16A’ flavour scores differed significantly only between the 18.3%SS pulp and the 11.9%SS pulp, although taster response was not clear-cut, and there was some suggestion of a relationship with base pulp and with sugar additions.

3.3. Effect of added sugar on volatile release

In general the headspace concentrations of flavour volatiles showed few significant changes in response to added sugar in both ‘Hort16A’ and ‘Hayward’ pulps. Statistical analysis (LDA) of the effect of sugar additions to both pulps, showed that headspace volatile concentrations differed between the controls and those with 4% added sugar (average of low and high dry matter) (Fig. 2a and b) but this was not significant at the 90%

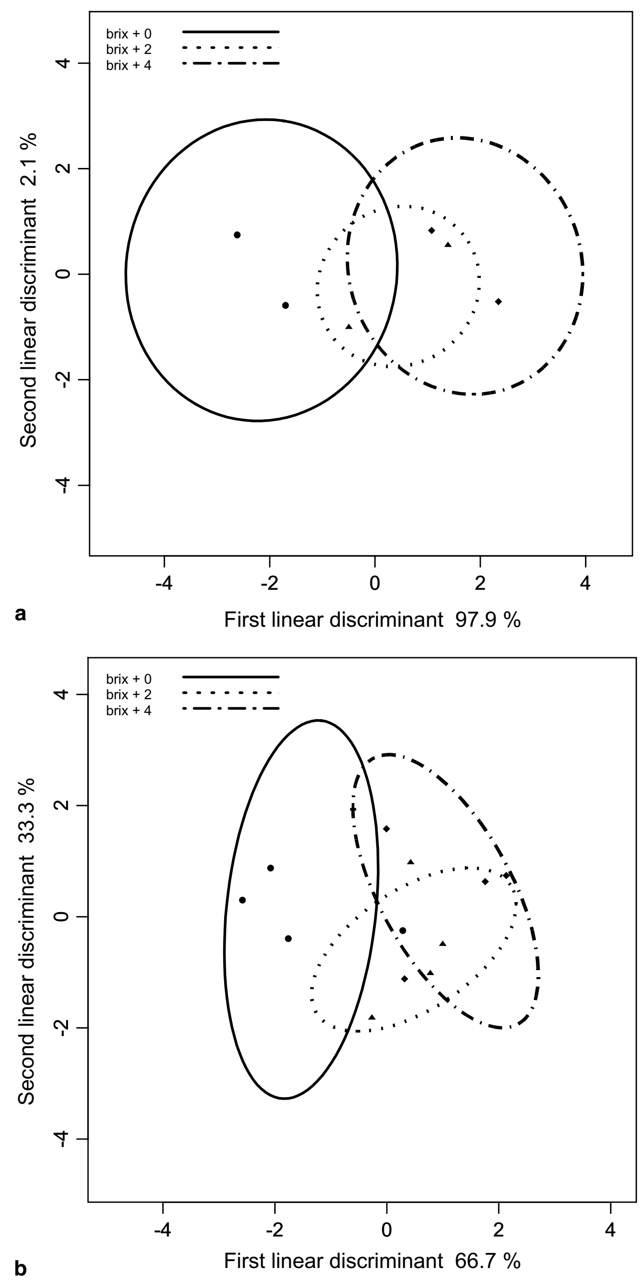


Fig. 2. LDA analysis of changes in volatile composition with added sugar to (a) ‘Hort16A’ pulps and (b) ‘Hayward’ pulps. The ellipses indicate the 95% confidence limits of log transformed data and (●) indicates control, (▲) control plus 2% sugar, (◆) control plus 4% sugar.

level. In 'Hort16A' (Fig. 2a), the pulps segregated primarily in the first discriminant, which was driven by increasing headspace concentrations of DMSO, acetic acid and 3-methylbutyl acetate, and 1,8-cineole (data not shown), although no one individual compound differed significantly. LDA analysis of the effect of added sugar in 'Hayward' pulps indicated that 4% addition of sugar segregated from controls, although again not significantly at the 90% level (Fig. 2b). Increasing ethyl butanoate and benzaldehyde were major drivers in segregating these two pulps. In addition, (*Z*)-3-hexenal, hexanal, (*Z*)-2-hexanal, (*E*)-2-hexenal, (*E*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol and acetone generally increased (not significant) with added sugar (data not shown).

3.4. Increasing acidity through addition of different acids

When buffered citrate was added to both 'Hayward' and 'Hort16A' low DM pulps, there were no differences in sensory quality compared with controls (Table 4). The effect of individual acids was ranked equally (generally a pH reduction of 0.2) and was perceived to be more acid than the control and buffered citrate pulps. All acids reduced perceived sweetness in 'Hayward' pulps, whereas only quinic and ascorbic acid caused 'Hort16A' pulps to be perceived as less sweet. All acid additions resulted in significantly higher scores for lemon flavours than the controls. Banana flavour scores declined when ascorbic and quinic acid were added to the pulps, with citric acid having only a moderate effect, which was not significantly different from controls or the other acids.

Addition of quinic acid caused a decline in perception of characteristic flavours in both pulps (Table 4). Astringency rating increased when acid was added to either pulp, and addition of acids to 'Hayward' pulps generally resulted in a stronger perception of astringency than when the same acids were added to 'Hort16A' pulps. Although an increase in grassiness was noted when acids were added to 'Hort16A' pulps, the effect was only significant with the addition of quinic acid.

3.5. Was there an effect of acid addition on headspace volatiles?

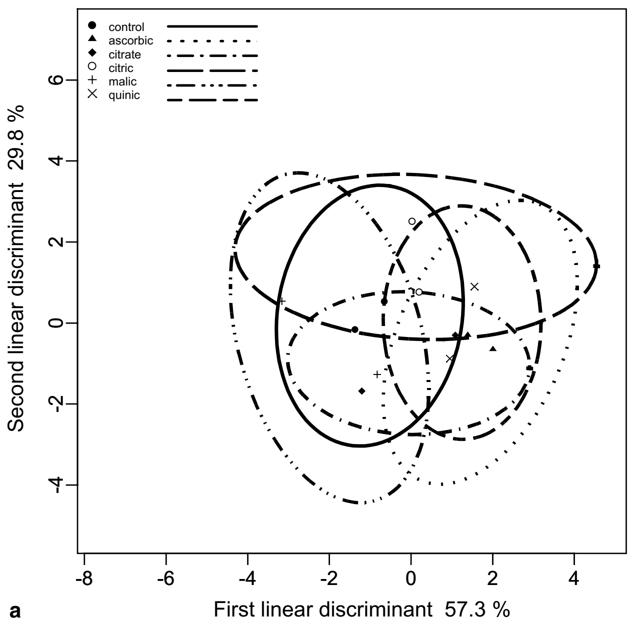
There were no statistically significant alterations to 'Hort16A' headspace volatiles with addition of any acid (Fig. 3a). Citric acid addition had the most impact on volatile concentrations with partial separation from the control occurring in the second linear discriminant. However it was difficult to define a major cause for this result. For example, 5 out of 7 esters increased with added citric acid–ethyl acetate, ethyl propanoate, ethyl 2-methylpropanoate (also affected by malic and quinic acids), ethyl butanoate and methyl butanoate. Three of these (ethyl acetate, ethyl propanoate and methyl butanoate) showed the same trend with ascorbic acid and citrate addition, indicating that it was not a pH effect.

Addition of acids to 'Hayward' pulps caused a significant alteration to head space volatiles (Fig. 3b). Total volatiles differed, with control and potassium citrate pulps containing the lowest total volatiles and malic acid pulps 2-fold more volatiles (NS, data not shown). Addition of acid resulted in changes to some medium chain

Table 4
Mean sensory score for attributes recorded by trained panellists (150 mm line scale) in pulp with various acid additions for (A) low dry matter 'Hayward' pulp, and (B) low dry matter 'Hort16A' pulp (n = 8; averaged over 2 days 'Hort 16 A' or 3 days 'Hayward' and tested for day effects)

	Control	Ascorbic	Citric	Malic	Quinic	Citrate	$LSD_{.05}$
(A) 'Hayward'[a]							
pH (measured)[b]	*3.7*	*3.6*	*3.5*	*3.5*	*3.5*	*3.8*	
Sweetness	82.1a	54.8b	63.9b	59.4b	53.2b	78.8a	9.4
Acidity	80.4b	106.4a	101.2a	105.5a	105.8a	81.2b	9.8
Lemon flavour	58.9b	75.9a	77.5a	76.3a	78.4a	60.6b	7.5
Banana flavour	45.8a	27.3b	33.4ab	26.4b	24.2b	44.8a	10.4
Characteristic 'Hayward' flavour	59a	48.6b	51ab	53.6ab	48.8b	62.7a	8.02
Grassy flavour	63.9a	66.8a	67.4a	69.8a	65.2a	60.1a	11.2
Astringency	62.7c	83.3ab	86.2a	86.4a	86.2a	68.2bc	10.3
(B) 'Hort16A'[a]							
pH (measured)[b]	*3.6*	*3.4*	*3.4*	*3.4*	*3.4*	*3.6*	
Sweetness	81a	56.2b	76.1a	70.2ab	53.1b	78.4a	18.1
Acidity	68.5b	98.3a	92.5a	94.2a	96.2a	75.2b	13.7
Lemon flavour	57.1b	75.9a	66ab	68.9a	70.0a	55.b	7.9
Banana flavour	49.5a	31.9b	36.7ab	36.5ab	29.1b	44.1ab	11.0
Characteristic 'Hort16A' flavour	67.1a	55.2ab	60.5ab	56.7ab	49.6b	64.1ab	11.5
Grassy flavour	28.0b	37.2ab	39.9ab	37.1ab	43.1a	38.5ab	9.4
Astringency	52.0b	62.6ab	66.1ab	63.2ab	69.5a	51b	11.0

[a] Numbers with the same letter following are not different at $p < 0.05$.
[b] Mean pH values were measured in the individual bulk pulp samples after acid addition.



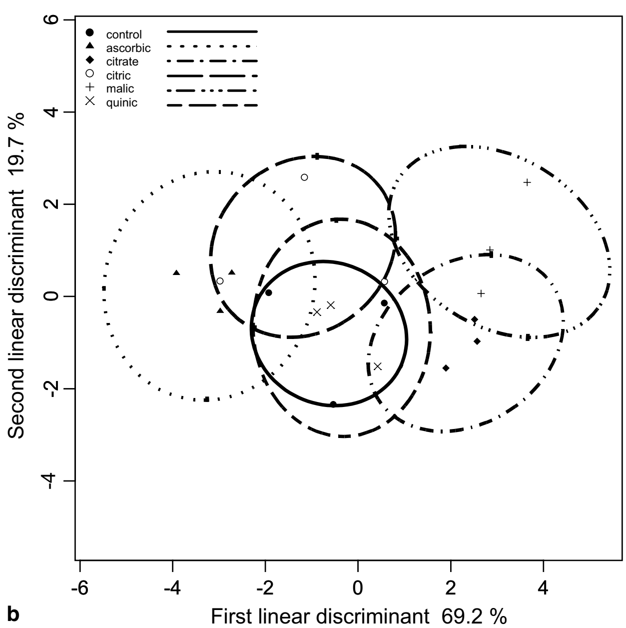


Fig. 3. LDA analysis of changes in volatile composition by adding acids to (a) 'Hort16A' pulps and (b) 'Hayward' pulps. The ellipses indicate the 95% confidence limits of log transformed data and (●) indicates control, (▲) control plus ascorbic acid, (◆) control plus potassium citrate, (○) control plus citric acid, (+) control plus malic acid, (×) control plus quinic acid.

length alcohols. *E*-3-hexen-1-ol was higher with addition of ascorbic, malic, and citric acids compared with control and citrate addition (data not shown). Addition of malic acid also resulted in significantly higher concentrations of hexanal ($p < 0.05$). 1-Hexanol and (*E*)-2-hexenal increased with addition of all solutions including potassium citrate although only the increase of 1-hexanol with citric acid was statistically significant ($p < 0.10$). Addition of ascorbic acid ($p < 0.10$) and citric acid ($p < 0.05$) also resulted in significantly higher concentrations of (*Z*)-3-hexenol.

Addition of malic acid resulted in a pulp with headspace volatiles that differed significantly from the controls. Addition of potassium citrate and citric acid also resulted in pulps with headspace volatiles that differed significantly from each other (Fig. 3b), but not from the remaining pulps. Methyl hexanoate, benzaldehyde and α-pinene were major drivers of discrimination between the control and malic acid pulps and ethyl and methyl butanoate were major drivers of discrimination between potassium citrate and citric acid pulps.

No change in volatile concentration could be associated with changes to characteristic 'Hort16A' or 'Hayward', lemon or banana flavours after acid additions. Where tasters described a significant reduction in sweetness in 'Hayward' pulps, 1-penten-3-ol, 1-pentanol, (*E*)-2-hexen-1-ol and (*E*)-3-hexen-1-ol showed an associated increase in headspace concentration, although none alone was significant (data not shown).

3.6. Addition of quinic acid is perceived differently to addition of malic and citric acid at the same pH

Preliminary experiments with trained tasters were carried out to compare perceived acidity and descriptors for each of the kiwifruit acids in solution at 1.5 g l^{-1} (data not shown). Quinic acid was described by panellists as chalky, aspirin-like, and fizzy. Citric acid was described as sharp, fresh and lemony, malic acid as lemony, tangy, bitter, sharp and green apple like, and ascorbic acid as soft, aspirin-like, bitter, astringent and lemon-like. Quinic acid was described by 67% of panellists as having a lingering aftertaste, while citric acid was described as sharp with no aftertaste. Some panellists found a sample of citric acid buffered with potassium citrate to have low acidity but a lingering slightly astringent aftertaste.

To determine if quinic acid was perceived as less palatable than the other acids in a pulp background, we conducted a consumer study with 40 untrained individuals tasting low DM 'Hayward' pulp. The acidity ratings for the different acids are presented on a labelled magnitude scale (Fig. 4). Consumers perceived quinic acid to be slightly more acidic than either citric or malic acids and significantly more acidic than controls.

4. Discussion

There are now consistent results demonstrating with both consumers and trained tasters that adding sugar to fruit pulps results in perceived increases in sweetness in pulps of three different fruit species—kiwifruit (Marsh et al., 2003; Rossiter et al., 2000) and mango (Malundo et al., 2001) and gold kiwifruit. We have also

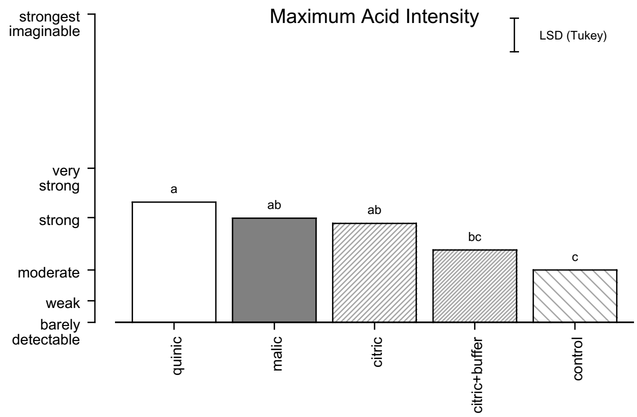


Fig. 4. The acidity rating of various acids and a buffered citrate solution presented to consumer panellists at 7 mM H^+/100 g and ranked on a 150 mm labelled magnitude scale ($n = 42$). (Columns with the same letter above are not different at $p < 0.05$.)

demonstrated that the increased sweetness can occur when pulps of either low or high starting sugar are used, and that the sugar or %SS of the pulp is the key feature to which tasters respond. Plotting the data from Table 3 (Fig. 5), showed that tasters did not reach an upper limit and the two pulps of different genetic backgrounds showed very similar taster response to SS level. The 'Hort16A' pulp always gave a slightly elevated sweetness score compared with the 'Hayward' pulp, suggesting that there was some other secondary feature that was important in determining sweetness for a taster. This is unlikely to be related to the type of sugar present, as using different sugars gave the same result as various mixes of sugars in 'Hayward' pulps (Marsh et al., 2003). Comparison of the composition of the starting material (Table 1) shows no real qualitative or quantitative differences in sugar composition. Djordjevic, Zatorre, and Jones-Gotmann (2004) have, however,

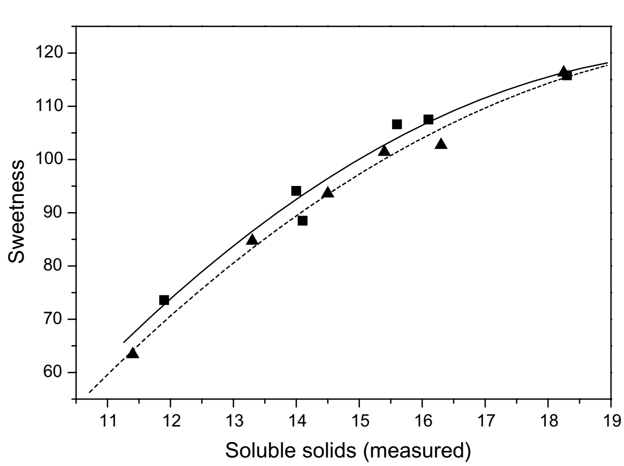


Fig. 5. Increased perception of sweetness by tasters in 'Hayward' (▲) and 'Hort16A' (■) pulps with increasing soluble solids concentration (SS). Data taken from Table 3.

recently demonstrated an odorant specific interaction in the detection of sucrose, suggesting that changes in volatile levels could lead to differences in sugar perception.

Acidity perception had a complex interaction with added acid or sugar in the different background pulps. Consumer panels rated 'Hayward' pulps with sugar added (11–14%SS) to a low sugar background as reducing perception of acidity (Rossiter et al., 2000), and trained tasters also perceived a reduction in acidity when sugar (2%) was added to 'Hayward' pulps of 14 and 15.5%SS (Marsh et al., 2003), but higher additions of sugar had no further effect. In contrast, this study with both low and high SS starting pulps (11% and 14%) showed no effect on acid perception due to added sugar with either genotype, a result in accordance with that of mango (Malundo et al., 2001). However, the background 'Hayward' pulps themselves differed and even though the SS concentration of the high DM pulp was the same as that of the low DM pulp with added sugar, a higher acid intensity was perceived. One of the measured differences between high and low DM base pulps was in quinic acid concentrations, and quinic acid in the experiments of Marsh et al. (2003) was similar to that of the low DM pulp used here. We suggest acid concentrations (primarily quinic) in the base pulp may provide the reason for different results in the literature.

Experiments with different genotypes of apple (Harker et al., 2002) indicated that tasters were able to distinguish acidity at differences as low as 0.08%TA. If this is translated into pH units we have added about 3 times more acid than this to our experimental pulps so perception of greater acidity is expected. Both consumer (Rossiter et al., 2000, this paper) and trained panels (Marsh et al., 2003, this paper) have described kiwifruit pulps with added acid as being more acid. In mango pulp (Malundo et al., 2001), various levels of added citric acid were resolved by multiple regression techniques; additions comparable to those normally encountered in mango increased ratings for sourness, bitterness and astringency. Hence there is now sound evidence that adding acids in the range present naturally in commercial fruits is detectable by tasters and affects taster perception.

Kiwifruit contain quinate as a major acid at levels similar to citrate levels (Okuse & Ryugo, 1981). Concentrations of quinate also vary between kiwifruit genotypes (Cheng et al., 2004). We found that quinic acid had a stronger impact on perception of acidity than citric, malic or ascorbic acids with both trained and consumer panels. Our findings are consistent with a free-choice profiling study (Rubico & McDaniel, 1992) in which the taste of malic, citric and quinic acids in a pH range of 3.1–3.6, were resolved into sour and astringent components by principal components analysis. PCA1 correlated with high acidity or sourness and ranked malic and citric acids higher than quinic, whereas PCA3 correlated with chalky, astringent, salty and sour flavors and ranked quinic acid higher than citric acid or malic acid (Rubico & McDaniel, 1992). Changes in some flavor notes may be a partly explained by the fact that quinic acid is a monoprotic acid so had a lesser effect on pH and a larger proportion of base per 7 mmoles H^+ compared with the triprotic citric or diprotic malic acid.

The increased perception of astringency with all the added acids, but not with potassium citrate in our experiments is clear confirmation of the suggestion by Lawless, Horne, and Giasi (1996) and Sowalsky and Noble (1998) that astringency is related to the hydrogen ion component of the acid and not the base. Our data also suggests that in a pulp matrix there is a threshold for perception of astringency. Adding sugar to the 'Hort16A' pulps had no effect on astringency. Astringency was reduced if sugar was added to the more astringent and acidic 'Hayward' pulps (see Table 3). By contrast, adding acid to both pulps did result in greater astringency for tasters. Clearly tasters do perceive different tastes based on the presence of different acids in fruit and there are several fruits such as kiwifruit (quinic acid) and grape (tartaric acid) where less common acids dominate acid composition.

The earlier experiments of Malundo et al. (2001) and Marsh et al. (2003) demonstrated that tasters perceive alterations to a number of flavour characters in fruit pulps when sugars or acids are added to the matrix. We measured the headspace volatiles to determine if there were significant changes that might relate to flavour perception. We confirmed that tasters perceive pulps with added sugar to be more banana-like and pulps with added acid to be less banana-like. Increases in esters together with reduction in some aldehydes and alcohols could contribute to the changes in perception of banana-like flavours. Friel et al. (2000) modelled the effect of sugar addition on the headspace concentration for a range of flavour volatiles. From this work, a 4% sugar addition to pulps would be predicted to increase the headspace concentration of some kiwifruit volatiles, e.g. (*E*)-2-hexenal (3%), 1-hexanol (6%), and ethyl butanoate (6%). Our pulps, however, showed little change in the release of esters with added sugars. An increase in release of some aldehydes did occur with added sugar e.g. hexanal, (*E*)-2-hexenal and benzaldehyde in 'Hayward', hexanal, decanal and benzaldehyde in 'Hort16A'. We propose that solution models do not apply easily to the fruit matrix, and that the altered banana and lemon flavours are due primarily to sugar and acid interactions in the mouth. More importantly, there were no changes in the ratings for characteristic flavours with added sugar.

Addition of acids could affect volatile release through pH shifts (possibly affecting enzymes present in the pulp) rather than added salt. Again some aldehydes and alcohols increased in the headspace with acid addition. Release of aldehydes with both sugar and acid addition suggests these compounds could result from a 'salting out' effect (Friel et al., 2000). However additional release of a number of alcohols with acid addition suggests this could be due to changes in kinetic conditions for alcohol dehydrogenases (Lai, Chandlee, & Scandalios, 1982).

5. Conclusions

Experiments designed to test the effect of realistic variations in sugar or acid, have demonstrated that tasters can be very sensitive to relatively small changes in the composition of fruit pulps. Pulps from two different genotypes primarily gave similar results. Some of the perceived changes in flavour characters may be associated with altered headspace composition, but the experiment was not extreme enough to unequivocally demonstrate this. A fruit matrix is much more complex than solutions or gels and models developed in these systems may not be very applicable to whole fruit. Use of standardised fruit pulps has enabled us to ask much more specific questions about the role of individual volatile compounds in formulating characteristic flavours for fruit in varying sugar and acid backgrounds. We suggest that the methodology, aligned with alternative approaches such as in mouth measurements, will have a useful application in testing taster preference and genotypic variation for both conventional and unusual flavours.

Acknowledgements

This research was funded by the New Zealand Foundation for Research, Science and Technology Contract No. C06X0006, C06X0213. We would like to thank Helen Boldingh and Mary Petley for help with chemical analyses.

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