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Conductivity at varying frequencies as a method for differentiating strawberry ripeness and association with colour acceptance of strawberry nectars

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Abstract

BACKGROUND: Identifying the best strawberries to produce colour stable nectars is a priority for the juice industry. Although riper strawberries produce nectars with better colour stability, variability between cultivars means that surface colour cannot be used as a single quality attribute to determine stability. Conductivity and bio-impedance measurements can be used to differentiate ripeness of strawberries. The commercially available PEF Control System (ELEA) can measure cell disruption by measuring conductivity at different frequencies. Updated software measured strawberry conductivity at 121 frequencies between 100 Hz and 1 MHz to determine whether conductivity at these frequencies could differentiate ripeness, and be compared with the colour acceptance and stability of nectars produced from these strawberries.

RESULTS: A high-low ratio (HLR) was calculated by dividing the conductivity at frequency 1 MHz by conductivity at 1 kHz. HLR could be used to separate five strawberry ripeness stages, with decreasing HLR associated with increasing ripeness. HLR was then compared with the colour of nectars produced from these strawberries. Although there was a good correlation between HLR and an acceptable colour to consumers on initial production ($r = -0.823$, $P < 0.001$) and after 12 weeks of storage (-0.759, $P < 0.001$), cultivars differed greatly in both HLR and colour stability. Additionally, HLR had a strong correlation with firmness.

CONCLUSION: The PEF Control System could be used to differentiate ripeness of strawberries by HLR, and therefore was associated with colour stability. However, no additional information on colour stability was gained from conductivity beyond what could already be deduced from differentiating ripeness based on surface colour.

Supporting information may be found in the online version of this article.

Keywords: frequency-dependant conductivity; strawberry nectar; colour stability; consumer acceptance; bio-impedance

INTRODUCTION

Strawberry nectars are problematic for producers and retailers because they undergo fast colour degradation, quickly changing from an attractive bright red colour to an unappealing brown colour, unacceptable to consumers.[1] The consumer acceptance of nectars can be derived from colour measurements to give an acceptance factor (AF),[1] which can be calculated on day of production and after 12 weeks to give a measure of colour stability.[2] Strawberries that are riper have been shown to produce nectars with higher colour stability.[3,4] Therefore, determination and differentiation of strawberry ripeness is crucial in the assessment of the suitability of raw material for nectar production. Many quality attributes change and develop during strawberry ripening including soluble solid content, acidity, aroma, antioxidant and phenol composition.[5-10] In previous studies, strawberry ripeness was either determined by days from colour set,[6,7] or by visual inspection of the surface colour.[5,8-10] Visual inspection is the standard method of determining strawberry ripeness in industry, where pickers examine the surface colour to determine when to harvest the fruit. However, with the development of modern systems,

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strawberries can occasionally have a surface colour that is not indicative of the quality expected from traditional ripening, with chemical and compositional attributes not correlating with surface colour.[11] Although generally surface colour and anthocyanin content are closely linked, in some cases, the surface colour is not completely indicative of the anthocyanin content of the strawberry.[11-13] Because anthocyanin content has been shown to be a good indicator of nectar colour stability,[2] methods other than examining surface colour would be useful to determine ideal ripeness of strawberries for production of colour stable strawberry nectars. This is particularity prudent when cultivar variability is taken into account, where strawberries with the same surface colour can possess very different colour stabilities.[3] Although strawberry nectar stability has been predicted by colour measurements after nectar production,[2] this prediction could only be determined after processing. It would be useful to have a method to determine colour stability prior to processing, so that strawberries that are unsuitable for nectar production are not processed unnecessarily, causing food waste.

Another parameter which changes during ripening is strawberry firmness, where strawberries become less firm during ripening due to breakdown of their cell walls.[14,15] The breakdown of cell walls has been shown to have a big impact in the change of electrical conductivity of fruit cells.[16] Electrical conductivity is the measure of how well an electric current flows through a material. In fruits and vegetables, conductivity is affected by a number of factors including chemical composition, tissue structure and firmness.[17] Previous research has shown that measuring electrical conductivity at a single frequency can differentiate ripeness of avocados[18] and degree of damage in strawberries.[19] Bio-impedance is related to conductivity by the equation $\sigma = K/Z$, where σ is the conductivity, K is the cell constant (dependent on the probe used to measure the conductivity) and Z is the impedance. Measuring impedance over a range of frequencies can be achieved by electrical impedance spectroscopy (EIS), which has been used extensively in the characterisation of fruit ripeness,[20] with nectarine,[21] banana,[22] mango[23] and persimmon ripeness[24] all determined. Of most interest, bio-impedance measurements have been shown to be able to differentiate strawberry ripeness.[25,26] These methods often rely on custom-built machinery and very advanced data processing, which are not practicable in an industrial setting, such as nectar production. Accordingly, the use of a commercially available device that could give simple readings, which could be associated with the ripening of strawberries, and therefore colour stability of strawberry nectars, would be of great interest.

The PEF Control System from ELEA (Quarkenbrücken, Germany) is a commercially available, portable, self-contained device that was designed to measure cell disintegration of fruits and vegetables after pulsed electric field (PEF) treatment. It achieves this by determining the cell integrity by measuring impedance as described by Angersbach *et al*.[16,27] The PEF controller generates a PCU (PEF Control Unit) measurement using high and low frequencies (1 kHz and 1 MHz) of a sample before and after PEF treatment.[28] The PCU requires the sample to be effectively compared to a 'normal' control sample.[29] Because there is no control sample to differentiate ripening, PCU alone cannot be used as a marker for ripeness differentiation. However, the principle that those samples with more intact cell wall structure will have different conductivities at varying frequencies than those that are more degraded (as during strawberry ripening) means the conductivity could be a starting point for differentiation of strawberry ripeness. This is supported by the fact that in tomatoes PEF treatment has been shown to mimic on-vine ripening,[30] and so riper strawberries should be analogous to fruits that have undergone PEF treatment.

The present study focused on using the PEF Control System to measure conductivity over a range of frequencies. To test this, the PEF software was modified to measure conductivity at 121 frequencies ranging between 100 Hz and 1 MHz. This work first investigated whether any of these conductivities were associated with changes in ripening, and therefore if any frequencies could be used to differentiate the ripeness stage of strawberries. Furthermore, the study also investigated whether the conductivity measurements were associated with the colour and colour stability of nectars produced from these strawberries, aiming to determine whether conductivity measurements could give information about nectar colour stability that cannot be deduced from surface colour alone.

MATERIALS AND METHODS

Strawberries

During the harvest season 2023, six samples of strawberries were collected from two different open field locations in Lower Austria. On 13 June 2023, cultivars 'Allegro', 'Sibilla' (hereafter referred to as Sibilla 1) and 'Malling Centenary' were harvested from fields in Zeislmauer. On 27 June 2023, the cultivar 'Sibilla' (hereafter referred to as Sibilla 2) was harvested from fields in Sighartskirchen, followed by 'Magnum' and 'Salsa' from the same location on 7 March 2023. These samples were sorted into five ripeness stages, depending on their surface colour: white (1), white–orange (2), orange (3), red (4) (normal ripeness) and dark red (5) (overripe) as shown in Fig. 1. This gave a total of 30 treatments (5 ripeness stages $\times$ 6 cultivar-location combinations). Strawberries were picked in the morning and a minimum of 0.5 kg of each treatment frozen at −18 °C within 4 h. Strawberries were kept frozen at −18 °C until nectar processing (approximately

Figure 1. Strawberry cultivar 'Allegro' sorted according to ripeness based on their surface colour. From left to right: white (1), white-orange (2), orange (3), red (4) (normal ripeness) and dark red (5) (overripe).

1 month). Fresh fruit (20 fruits per treatment) were kept for conductivity, size, weight and firmness measurements.

Methods

Preparation of nectars

Defrosting of strawberries and puree preparation. Frozen strawberries were thawed for 24 h at room temperature, and then processed into puree by milling with a 1-mm sieve (Reber, Luzzara, Italy).

Nectar preparation. Nectars were produced on a lab-scale as described in Murray *et al.*[2] Purees were mixed with necessary quantities of water, citric acid and sugar to produce nectars that contained 40% strawberry puree content, 15 °Brix and 7.0 g L^{-1} titratable acidity and were then homogenised with a hand blender (Philips, Drachten, The Netherlands). Next, 140 g ± 1 g of nectar was weighed into minimum 12 glass jars (212 mL), which were sealed with a screw lid and pasteurised (85 °C 20 min) in a water bath (Royal Catering, Berlin, Germany). Nectars were stored at room temperature (20° ± 3 °C) in the dark for 12 weeks.

Conductivity measurements

Conductivity was measured using a modified PEF Controller (ELEA, Quakenbrück Germany) based on Angersbach.[16,27] The PEF Control Unit was updated with specifically designed software which measured conductivity over a range of 121 frequencies from 100 to 1 000 000 Hz. Strawberries were cut in half lengthways, two measurements taken from each half by fully inserting the prongs into the fruit of the flat halves, avoiding the central void (Fig. 2). Four measurements were taken per strawberry (Fig. 3), with 10 strawberries measured per treatment (i.e. a total of 40 measurements per treatment).

High-low ratio. Dividing the conductivity (in mS cm^{-1}) of a sample at 1 MHz (high frequency) by the conductivity at 1000 Hz (low frequency) gave a dimensionless value, which was named the high-low ratio (HLR) as shown in eq. 1:

$$\mathrm{HLR} = \text{conductivity at 1 MHz}/\text{conductivity at 1000 Hz} \qquad (1)$$

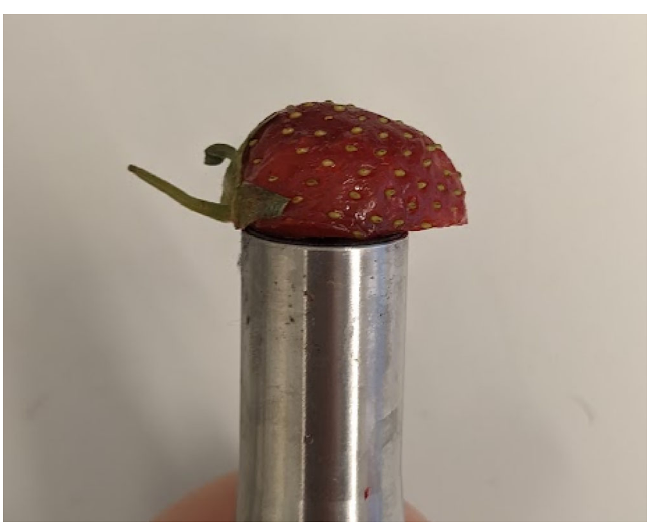

Figure 2. Placement of the strawberry sample on the prongs of the probe of the PEF Control Unit. Sliced in half and then fully inserted.

Figure 3. Site of the four measurements from each strawberry, avoiding the central void.

Physical and chemical analysis

Total soluble solids (°Brix) were measured with a hand-held refractometer (N-20; Brix 0–20%; ATAGO, Tokyo, Japan). pH values were determined using a pH meter (MultilineP4; WTW, Weilheim, Germany) and pH electrode (SenTix 41-3; WTW). Firmness values were obtained using a penetrometer (AFG 500N, stamp 8 mm; Mecmesin, Slinfold, UK) measuring 10 fresh strawberries from each treatment, on two sides (20 measurements total) and multiplied by 2 to express firmness in kg cm^{-2}. It was not possible to measure firmness of Sibilla 2 because of the small sample size. Titratable acidity was determined by titration to an endpoint of pH value 7.0 (0.1 N NaOH Titrisol®; Merck KGaA, Darmstadt, Germany) and multiplied by the acidity factor of tartaric acid (0.75) to express acidity as g L^{-1}.

Colour measurements

CIELAB-system colour components L^* (lightness), a^* (red-green) and b^* (yellow-blue) were measured using a CM-5 spectrophotometer (spectrophotometric method, D65, 30 mm 10°, reflection measurement, gloss excluded; Minolta, Osaka, Japan). C^* (Chroma) and $h°$ (hue angle) were calculated as previously reported.[31] Acceptance factor (AF) was calculated as $\mathrm{AF} = \frac{a^*}{h}$ as previously reported.[1] All nectars were measured after pasteurisation on day of production and then every 4 weeks for 12 weeks. Each sample was measured twice, in duplicate. The colour stability was quantified with different indicators of colour stability: AF0 is the AF after initial production and AF12 is the AF after 12 weeks of storage as described in Murray *et al.*[2]

Statistical analysis

Statistical analysis [analysis of variance (ANOVA), Tukey's honestly significant difference, curve fitting, regression analysis and pearson correlations] was carried out using SPSS, version 26 (IBM Corp., Armonk, NY, USA). Conductivity graphs were analysed with Excel (Microsoft, Redmond, WA, USA). ANOVA followed by Tukey's test was undertaken to determine significant differences ($P < 0.05$) between ripeness stages for different frequencies, HLR, AF0, AF12 and firmness. Pearson correlations were calculated between HLR and AF0, AF12, firmness and colour components.

RESULTS

Conductivity at all frequency points for all ripeness stages

Figure 4 shows the average conductivities across all cultivars at all frequencies, for each of the different ripeness stages. At low frequencies, the lowest ripeness stages had the lowest conductivities, and higher ripeness stages had higher conductivity. By contrast, at high frequencies, the conductivity was lower for riper fruit, and higher for less ripe fruit.

Frequencies for differentiation of ripeness stages

Table 1 shows the average conductivity at selected frequencies for each ripeness stage, averaged across all cultivars. Statistical separation ($P < 0.05$) of ripeness stages was possible at very low and very high frequencies. At low frequency (1000 Hz), all ripeness stages could be significantly separated. The separation of ripeness stages was better at 1000 Hz than at 100 Hz, and this could be because 1000 Hz is the recommended low frequency of the PEF Control System and, consequently, the instrument is more reliable at this frequency than at lower frequencies or because of interference with the matrix. At high frequency (999 999.812; hereafter rounded to 1 MHz) statistical difference was found only between ripeness stages 1 and 2 and the other stages (3–5). The conductivities of the individual cultivars at these frequencies can be found in the Supporting information (Table S1). There was no uniform differences within cultivars; however, in all cases, there was a trend for low ripeness having lower conductivities at lower frequencies, and higher conductivities at higher frequencies.

It was found that conductivities from frequencies in the range of 17 113–116 591 Hz were particularly unsuitable for differentiating ripeness stages, with very little difference found between the ripeness stages. This is exemplified in Table 1, where, at a frequency of 50 118.715 Hz, there was no significant difference in the conductivities between four of the ripeness stages. ANOVA analysis of the conductivity of the ripeness stages at all frequencies can be found in the Supporting information (Table S2).

HLR for the assessment of nectar colour stability

The conductivity trends outlined above in the section on 'Frequencies for differentiation of ripeness stages' led to the development of the HLR (see section 'High-low ratio'). The data shown in Table 1 demonstrate that the HLR could be used to significantly differentiate all five different ripeness stages, with ripeness stages 4 and 5 having the lowest HLR.

Table 2 shows the correlations between the average HLR of each treatment and the colour components of nectars produced from these strawberries, on the day of nectar production and after 12 weeks of nectar storage. HLR was strongly positively correlated with L^* and negatively correlated with a^*, both before and after nectar storage. Therefore, samples with higher HLR were lighter and less red, both when initially produced and after 12 weeks of storage. HLR showed no significant correlation with b^* before storage. The L^*, a^*, b^*, C^* and $h°$ of the nectars before and after 12 weeks of storage can be found in the Supporting information (Table S3).

HLR was highly correlated with the AF, both before (AF0) and after storage (AF12). The correlation was stronger for the initial

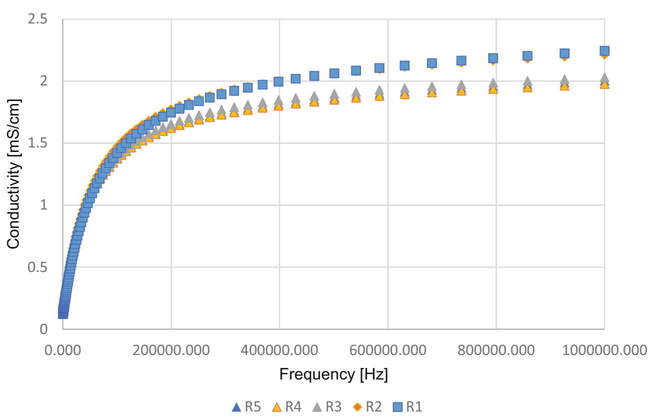

Figure 4. The average conductivity at each ripeness stage based on surface colour, where R1 is white, R2 is white–orange, R3 is orange, R4 is red (normal ripeness) and R5 is dark red (overripe), for all 121 frequencies.

Table 2. Pearson correlations between the high-low ratio (HLR) of strawberries and colour components of nectars before and after 12 weeks of storage

Colour component	HLR correlation with fresh nectar	HLR correlation with nectar stored for 12 weeks
L^*	0.808**	0.693**
a^*	−0.807**	−0.774**
b^*	−0.277	0.666**
C^*	−0.762**	−0.508**
$h°$	0.718**	0.777**
Acceptance factor	−0.821**	−0.760**

** $P < 0.01$.

Table 1. The mean ± SD of the conductivity (mS cm^{-1}) of strawberries at each ripeness stage at selected frequencies, and the high-low ratio (HLR) (conductivity at frequency 1 kHz/conductivity at 1 MHz)

	Frequency	100.000 Hz	1000.000 Hz	50 118.715 Hz	999 999.812 Hz	HLR
All cultivars	White (1)	0.122 ± 0.018 a	0.149 ± 0.018 a	1.055 ± 0.103 a	2.243 ± 0.331 b	15.137 ± 1.725 e
	White/orange (2)	0.129 ± 0.020 a	0.162 ± 0.021 b	1.118 ± 0.108 b	2.211 ± 0.336 b	13.773 ± 1.918 d
	Orange (3)	0.137 ± 0.032 b	0.173 ± 0.039 c	1.075 ± 0.130 a	2.030 ± 0.335 a	11.947 ± 1.817 c
	Red (4)	0.145 ± 0.290 c	0.182 ± 0.031 d	1.073 ± 0.106 a	1.977 ± 0.332 a	11.015 ± 1.737 b
	Dark red (5)	0.161 ± 0.045 d	0.199 ± 0.055 e	1.079 ± 0.126 a	2.001 ± 0.291 a	10.435 ± 2.171 a

Note: Different lowercase letters (vertical) illustrate significant differences ($P < 0.05$).

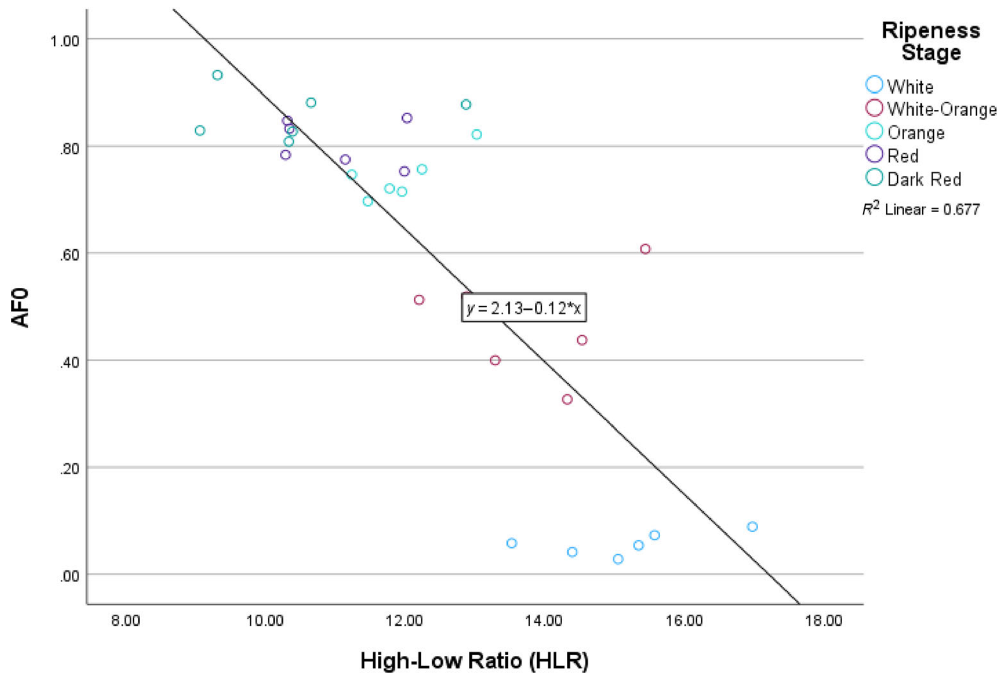

Figure 5. The average high-low ratio (HLR) of each strawberry treatment plotted against the acceptance factor after initial production (AF0) of nectars produced from the same strawberries. Different colours represent different ripeness stages.

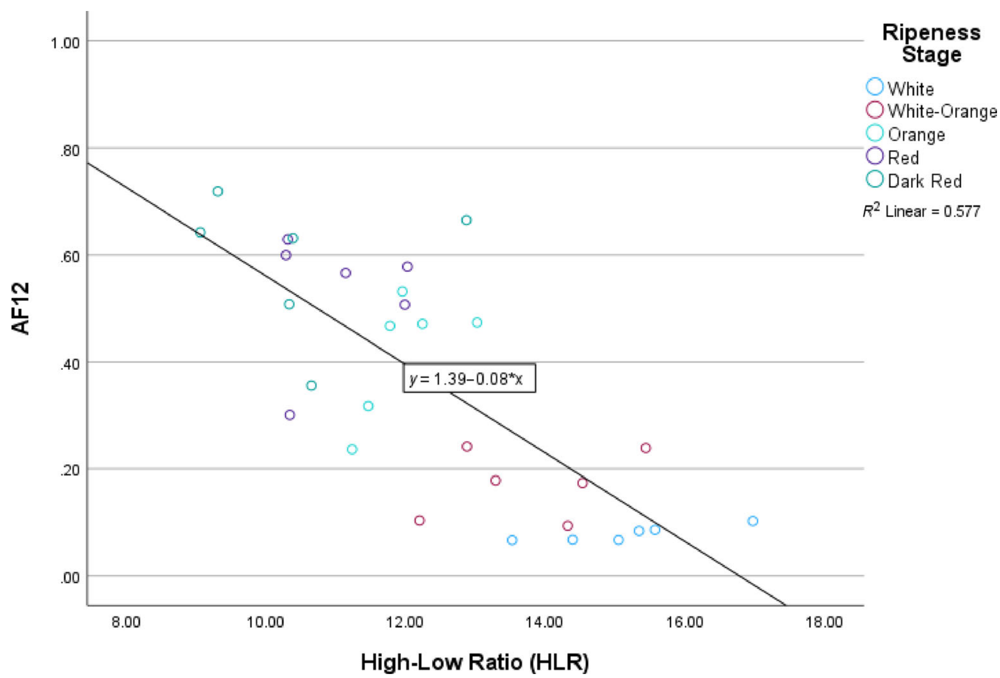

Figure 6. The average high-low ratio (HLR) of each strawberry treatment plotted against the acceptance factor of nectars produced from the same strawberries after 12 weeks of storage (AF12). Different colours represent different ripeness stages.

colour than the stored colour. Figure 5 shows the relationship between HLR and AF0; the regression was strong and significant ($R^2 = 0.677$, $P < 0.001$). Figure 6 shows the relationship between the HLR and AF12, which gives an indication of colour stability. The relationship was also significant, but not as strong as with AF0, with a regression of $R^2 = 0.577$ ($P < 0.001$).

The nectars were sorted into five groups by descending HLR, as shown in Table 3. Nectars produced from strawberries with HLR above 13.5 (HLR groups 1 and 2) had statistically worse AF0 and AF12 than the other samples, possessing a colour that was below the level of acceptability both when first produced and after storage. By contrast, strawberries with an HLR below 10.5 (HLR group 5) had an excellent colour when first produced, and a significantly higher AF12 than all the HLR groups below this level. Strawberries with a HLR between 10.5 and 13.5 generally produced nectars that were an acceptable colour when first produced, but had mixed stability, and so HLRs in this range could not accurately determine colour stability.

Table 3. Nectar samples sorted into groups as defined by the mean high-low ratio (HLR) of the strawberries the nectars are produced from, and the mean acceptance factor of these nectars when initially produced (AF0) and after 12 weeks of storage (AF12)

HLR group	HLR range	Samples	Number of samples	AF0	AF12
1	> 15	Sibilla 2 ripeness 1	5	0.170 ± 0.225 a	0.115 ± 0.063 a
		Salsa ripeness 1			
		Sibilla 2 ripeness 2			
		Malling centenary ripeness 1			
		Allegro ripeness 1			
2	13.5–15	Malling centenary ripeness 2	4	0.215 ± 0.177 a	0.100 ± 0.045 a
		Sibilla ripeness 1			
		Sibilla ripeness 2			
		Magnum ripeness 1			
3	12–13.5	Salsa ripeness 2	7	0.678 ± 0.184 b	0.387 ± 0.200 b
		Sibilla 2 ripeness 3			
		Allegro ripeness 2			
		Sibilla 2 ripeness 5			
		Malling centenary ripeness 3			
		Magnum ripeness 2			
		Sibilla 2 ripeness 4			
4	10.5–12	Sibilla ripeness 4	7	0.755 ± 0.058 bc	0.425 ± 0.116 b
		Allegro ripeness 3			
		Salsa ripeness 3			
		Sibilla ripeness 3			
		Magnum ripeness 3			
		Malling centenary ripeness 4			
		Magnum ripeness 5			
5	< 10.5	Malling centenary ripeness 5	7	0.836 ± 0.046 c	0.574 ± 0.128 c
		Magnum ripeness 4			
		Sibilla ripeness 5			
		Salsa ripeness 4			
		Allegro ripeness 4			
		Salsa ripeness 5			
		Allegro ripeness 5			

Note: Different lowercase letters (vertical) illustrate significant differences ($P < 0.05$).

Impact of cultivar on HLR and colour stability

Table 4 shows the individual average HLR for each of the different treatments (defined by the cultivar and ripeness stage), alongside their AF0 and AF12. For all cultivars, there was always a significant separation between the most unripe (1 and 2) and most ripe stages (4 and 5), but, for some cultivars, not all the ripeness stages could be separated. Different cultivars had different ranges for the HLR, suggesting the cultivar of the strawberry affected conductivity. Within cultivars, the AF0 of each ripeness stage was significantly different, with the more ripe stages having a higher AF0. This was also the case for AF12 (except for the sample Sibilla 1, which had no significant difference between ripeness 4 and 5). There was a large difference in colour stability between the different cultivars. The sample with the highest individual AF12 was ripeness 5 from cultivar 'Salsa'.

Samples of the same ripeness stages but from different cultivars were significantly different. HLR could not be used as a parameter to distinguish colour stable fruits from unstable fruits of the same ripeness stage. This is exemplified by the fact that, when averaged across all ripeness stages, 'Allegro' had a significantly higher AF12 (0.416) than 'Magnum' (0.212) ($t_{38} = 3.6$, $P = 0.001$), despite no significant difference between their HLRs ($t_{398} = -1.1$, $P = 0.260$). Of particular note, cultivar 'Allegro' at ripeness Stage 3 (orange) had a significantly better AF12 (0.532) than the ripeness stage 5 (dark red) of 'Magnum' (0.355) (t_6 =124.1, $P < 0.001$), despite the 'Allegro' ripeness Stage 3 (orange) having a significantly higher HLR (15.196 *vs.* 13.509) (t_{76}) = 3.0, $P = 0.003$) and a significantly lower AF0 (0.715 *vs.* 0.879) ($t_6 = -76.0$, $P < 0.001$).

Cultivar was not the only parameter to consider, with harvest date and origin having an impact. The two samples of 'Sibilla' had significantly different HLRs ($t_{398} = -6.7$, $P < 0.001$). Sibilla 2 had a higher HLR (14.06) than Sibilla 1, which should mean it would be less colour stable, althoughthere was no significant difference in AF0 ($t_{38} = -1.3$, $P = 0.185$) or AF12 ($t_{38} = -$ 236 1.7, $P = 0.093$). Ripeness stage 4 of Sibilla 2 had a significantly higher AF12 than the ripeness stage 5 of Sibilla 1 ($t6 = 25.2$, $P < 0.001$), despite the significantly higher HLR ($t_{78} = 4.9$, $P < 0.001$). Additionally, although there was no significant difference between either AF0 and AF12 when averaged across all ripening stages, the AF12 of grades 4 and 5 were significantly higher for Sibilla 1 than for Sibilla 2.

HLR association with physical and chemical parameters

Total soluble solids and titratable activity

The total soluble solids (TSS) and titratable acidity (TA) of the strawberry variants are shown in Table 5, and the correlations with

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Table 4. The mean and standard deviation for the high-low ratio (HLR) (conductivity at frequency 1 kHz/conductivity at 1 MHz) and the acceptance factor of nectars produced from the same strawberries when they are initially produced (AF0) and after 12 weeks of storage (AF12), at each ripeness grade, for each strawberry sample

		Allegro	Malling centenary	Sibilla 1	Sibilla 2	Magnum	Salsa
HLR	White (1)	15.04 ± 1.34 eBC	15.34 ± 0.90 cC	14.39 ± 1.33 cAB	16.97 ± 1.48 dD	13.52 ± 1.59 cA	15.57 ± 1.49 dC
	White/orange (2)	12.88 ± 1.48 dAB	14.53 ± 1.99 cCD	14.32 ± 1.47 cC	15.43 ± 1.66 cD	12.20 ± 1.50 bA	13.29 ± 1.37 cB
	Orange (3)	11.95 ± 1.82 cAB	12.24 ± 1.61 bAB	11.46 ± 2.16 bA	13.02 ± 1.59 bB	11.23 ± 1.24 abA	11.77 ± 1.88 bcA
	Red (4)	10.29 ± 1.08 bA	11.14 ± 1.86 abAB	11.99 ± 1.28 bB	12.02 ± 1.49 aB	10.34 ± 1.51 aA	10.31 ± 2.05 bA
	Dark red (5)	9.06 ± 1.48 aA	10.39 ± 2.54 aBC	10.33 ± 1.48 aBC	12.87 ± 1.62 abD	10.65 ± 2.00 aC	9.31 ± 1.53 aAB
	All ripeness grades	11.84 ± 2.53 AB	12.72 ± 2.66 C	12.50 ± 2.22 BC	14.06 ± 2.41 D	11.59 ± 1.95 A	12.05 ± 2.78 ABC
AF0	White (1)	0.028 ± 0.000 aA	0.054 ± 0.001 aC	0.041 ± 0.000 aB	0.089 ± 0.000 aF	0.057 ± 0.001 aD	0.073 ± 0.000 aE
	White/orange (2)	0.519 ± 0.004 bE	0.439 ± 0.003 bC	0.324 ± 0.003 bA	0.607 ± 0.002 bF	0.512 ± 0.002 bD	0.400 ± 0.001 bB
	Orange (3)	0.715 ± 0.004 cB	0.757 ± 0.004 cD	0.692 ± 0.006 cA	0.826 ± 0.005 cE	0.746 ± 0.001 cC	0.721 ± 0.002 cB
	Red (4)	0.783 ± 0.002 dB	0.776 ± 0.006 dB	0.754 ± 0.002 dA	0.851 ± 0.003 dD	0.830 ± 0.003 dC	0.849 ± 0.005 dD
	Dark red (5)	0.829 ± 0.006 eB	0.827 ± 0.006 eB	0.796 ± 0.014 eA	0.881 ± 0.004 eC	0.879 ± 0.002 eC	0.934 ± 0.003 eD
	All ripeness grades	0.575 ± 0.301 A	0.570 ± 0.300 A	0.522 ± 0.300 A	0.651 ± 0.305 A	0.605 ± 0.309 A	0.595 ± 0.326 A
AF12	White (1)	0.067 ± 0.000 aA	0.084 ± 0.001 aB	0.068 ± 0.001 aA	0.101 ± 0.001 aC	0.066 ± 0.001 aA	0.086 ± 0.001 aB
	White/orange (2)	0.241 ± 0.000 bF	0.173 ± 0.001 bC	0.093 ± 0.001 bA	0.236 ± 0.003 bE	0.103 ± 0.001 bB	0.178 ± 0.000 bD
	Orange (3)	0.532 ± 0.002 cD	0.471 ± 0.004 cC	0.318 ± 0.002 cB	0.473 ± 0.001 cC	0.235 ± 0.001 cA	0.468 ± 0.002 cC
	Red (4)	0.600 ± 0.002 dE	0.566 ± 0.003 dC	0.505 ± 0.004 dB	0.576 ± 0.003 dD	0.301 ± 0.001 dA	0.626 ± 0.005 dF
	Dark red (5)	0.642 ± 0.004 eD	0.628 ± 0.006 eC	0.505 ± 0.005 dB	0.663 ± 0.005 eE	0.355 ± 0.002 eA	0.717 ± 0.004 eF
	All ripeness grades	0.416 ± 0.300 B	0.385 ± 0.222 AB	0.298 ± 0.195 AB	0.408 ± 0.216 B	0.212 ± 0.114 A	0.415 ± 0.253 B

Note: These are referred to by their cultivar where there are two samples of the same cultivar (Sibilla) 1 and 2 refers to the first and second harvested samples. Different lowercase letters (vertical) illustrate significant differences ($P < 0.05$). Different uppercase letters (horizontal) illustrate significant differences between cultivars ($P < 0.05$).

HLR, AF0 and AF12 are shown in Table 6. There was no significant correlation between the TSS and the AF0, AF12 or the HLR. Ripeness stage had a lower impact on TSS than the cultivar, with cultivar 'Magnum' having the highest TSS. Harvest date and origin also had a large impact on TSS, with the TSS of Sibilla 2 being higher than Sibilla 1. The TA had a significant positive correlation with HLR, and a significant negative correlation with AF0 and AF12. Within the cultivars, the TA decreased with ripening stage and, although there was no significant difference between most of the cultivars, Sibilla 2 had a significantly higher TA than all the other treatments.

Firmness

The average firmness for the different ripeness stages are shown in Table 5, and the correlation with HLR is shown in Table 6. HLR and firmness had a significant positive correlation; therefore, the lower the HLR, the lower the firmness. The firmness decreased with ripening stage, but, although the HLR could significantly separate the different ripening stages, firmness could only significantly separate the white (ripeness 1) and white–orange (ripeness 2) strawberries, from the other ripeness stages. The firmness was highly negatively correlated with AF0 ($r = -0.926$, $P < 0.001$), but was less well correlated with AF12 ($r = -0.716$, $P < 0.001$).

DISCUSSION

The separation of ripeness stages by the conductivity at varying frequencies is in agreement with the study by Angersbach *et al.*,[16] which found that at lower frequencies less disrupted cells (as found in less ripe strawberries[14]) have a lower conductivity, and, at higher frequencies, the more disrupted cells have a lower conductivity. The use of a ratio at 1 kHz and 1 MHz is particularly suitable for using the PEFControl unit to determine ripeness because those are the frequencies utilised in the PCU calculation[28] which is in-built into the instrument. This requires only two measurements to be taken rather than the whole spectra of frequencies, saving data acquisition time and simplifying data analysis. Simplified data analysis makes the HLR more practical for industrial application, especially in comparison to previous work, which used advanced computation methods.[25,26] The present study supports previous research showing that the electrical properties of strawberries change during ripening, and these changes can be used to determine strawberry ripeness.[25,26] Ibba *et al.*[26] used bio-impedance to separate strawberries into 'ripe' and 'underripe'. Our findings support that electrical properties are particularly adept at separating underripe strawberries from ripe or overripe strawberries, as shown by the clear difference in HLR between ripening stages 1 and 2, compared to stages 4 and

Table 5. Mean firmness, total soluble solids and titratable acidity at each ripeness grade, for each sample of strawberry

		Allegro	Malling centenary	Sibilla 1	Sibilla 2	Magnum	Salsa
Firmness (kg cm^{-2})	White (1)	2.09 ± 2.11 bA	1.50 ± 1.38 bA	1.86 ± 1.21 cA	–	2.30 ± 3.28 bA	1.48 ± 1.51 bA
	White/orange (2)	0.39 ± 0.35 aA	0.42 ± 0.18 aA	0.91 ± 0.80 bB	–	0.30 ± 0.17 aA	0.50 ± 0.23 aA
	Orange (3)	0.19 ± 0.10 aA	0.28 ± 0.15 aAB	0.30 ± 0.15 aB	–	0.21 ± 0.11 aAB	0.22 ± 0.07 aAB
	Red (4)	0.16 ± 0.09 aA	0.20 ± 0.09 aA	0.21 ± 0.12 aA	–	0.15 ± 0.07 aA	0.23 ± 0.13 aA
	Dark red (5)	0.17 ± 0.09 aA	0.15 ± 0.07 aA	0.17 ± 0.05 aA	–	0.16 ± 0.05 aA	0.15 ± 0.08 aA
	All ripeness grades	0.60 ± 1.21 A	0.51 ± 0.80 A	0.69 ± 0.91 A	–	0.62 ± 1.67 A	0.51 ± 0.84 A
Total soluble solids (TSS) (°Brix)	White (1)	5.9	5.8	5.7	8.0	8.5	8.1
	White/orange (2)	6.1	5.9	6.1	8.0	9.0	8.2
	Orange (3)	6.0	6.0	6.2	8.8	8.8	7.0
	Red (4)	7.1	7.0	6.6	8.1	9.6	8.4
	Dark red (5)	6.4	6.7	6.9	7.5	10.1	9.2
	All ripeness grades	6.3 ± 0.4 A	6.3 ± 0.5 A	6.3 ± 0.4 A	8.1 ± 0.4 B	9.2 ± 0.6 C	8.2 ± 0.7 B
Titratable acidity (TA) (g L^{-1})	White (1)	10.7	9.8	8.0	17.0	13.8	10.3
	White/orange (2)	10.0	7.9	8.5	15.2	9.8	9.8
	Orange (3)	8.0	7.4	7.4	12.5	6.5	6.5
	Red (4)	6.8	5.9	6.1	10.2	5.6	5.6
	Dark red (5)	6.1	5.4	5.9	9.8	5.0	5.0
	All ripeness grades	8.3 ± 1.9 A	7.3 ± 1.6 A	7.2 ± 1.0 A	12.9 ± 2.9 B	8.8 ± 2.8 A	7.4 ± 2.2 A

Note: Different lowercase letters (vertical) illustrate significant differences ($P < 0.05$). Different uppercase letters (horizontal) illustrate significant differences between cultivars ($P < 0.05$).

Table 6. Pearson correlations between firmness, total soluble solids and titratable acidity of strawberries with the high-low ratio (HLR) of strawberries, and the acceptance factor of nectars produced from these strawberries on day of production (AF0) and after 12 weeks of storage (AF12)

	Correlation with HLR	Correlation with AF0	Correlation with AF12
Firmness (kg cm^{-2})	0.760**	−0.926**	−0.716**
Total soluble solids (TSS) (°Brix)	−0.241	0.295	0.071
Titratable acidity (TA) (g L^{-1})	0.784**	−0.563**	−0.555**

** $P < 0.01$.

5. This separation allowed the HLR to differentiate underripe strawberries from those at a ripeness stage suitable to produce nectars with acceptable colour.

Colour stability of the nectars was strongly associated with ripening, as defined by surface colour. Within each cultivar, both the initial nectar colour, and the nectar colour after storage, improved with increasing ripeness stage. This is in agreement with previous studies that found overripe strawberries (dark red surface colour) produced nectars with a more stable colour.[4] Despite this, surface colour alone could not be used to predict the colour stability of nectars produced from these strawberries. As described in previous research,[4] cultivar variability had a large impact on the colour stability of nectars, with less ripe strawberries from some cultivars having better colour stability than more ripe fruit from other cultivars. Because the ripeness stage was associated with colour stability, and HLR was associated with the ripeness stage, the HLR was also associated with colour stability. The HLR had a good correlation with both the AF0 and AF12, but was better correlated with initial nectar colour than colour after storage. This suggests that the HLR was better at identifying which cultivar-ripening stage are more suitable for producing nectars of an acceptable initial colour, rather than those with good colour stability. HLR was also highly positively correlated with L^* and negatively correlated with a^*, and so strawberries with low HLR have a darker and more red colour, which lead to more colour stable nectars.[2]

In agreement with previous studies, firmness decreased with increasing ripeness stage,[6,25] although only the least ripe strawberries could be statistically differentiated from the other ripening stages. Firmness was strongly correlated to the colour of nectars, both before and after storage. Firmness was also strongly correlated with the HLR, because both parameters are a measure of cell disintegration of strawberries.[14,16] HLR had a better correlation with the AF12 than the firmness, and was also more effective at separating ripeness stages. Therefore, although both firmness and conductivity analyses determine ripening through the breakdown of the cell wall, conductivity is a more effective tool at determining the ripeness of strawberries for nectar production than firmness.

Previous studies found that TSS increased with ripening stage until full ripeness, with a reduction sometimes occurring once the fruit becomes too overripe.[5,6] In the present study, this trend was generally observed within cultivars, with white fruit (ripeness 1) having the lowest TSS, and the riper fruit (4 and 5) having the highest. The cultivar and harvest conditions had a greater impact on the TSS than ripening stage. In agreement with previous studies,[6] the TA decreased with ripeness stage, which would explain the strong correlation observed with HLR.

CONCLUSIONS

The PEF Control System from ELEA was able to separate the different ripeness stages of strawberry samples, based on a ratio of the conductivity at frequencies of 1 kHz and 1 MHz (HLR). The HLR was particularly adept at identifying strawberries that were below acceptable ripeness for producing nectars. Above a value of 13.5, strawberries produced nectars with unacceptable colour. HLR had a particularly strong relationship with the initial acceptance factor of nectars (AF0), but was less strongly correlated with the colour of nectars after storage (AF12). Despite this, HLR could provide a value (10.5) below which strawberries were suitable to produce nectars that had a good colour after storage. HLR was well correlated with firmness because both measure cell disintegration, but HLR was more adept at differentiating ripeness stages than firmness.

When comparing strawberries that only differed in their ripeness stage (i.e. same cultivar, harvest date and origin), the colour stability of nectars increased with increasing ripeness. However, when comparing strawberries that were from different cultivars and harvests, the ripeness stage alone could not be used to predict colour stability. Less ripe strawberries of some cultivars produced more colour stable nectars than some more ripe strawberries from other cultivars, with the ripeness 3 (orange) of Allegro, Salsa and Malling Centenary all having a better colour after storage than the ripeness 5 (dark red) of Magnum. Although there were large differences in HLR between the different cultivars, these differences could not be used to determine colour stability. The HLR therefore provided no extra information on colour stability beyond what could already be determined from surface colour.

The PEF Control system is a commercially available device that would be of use in differentiating the different ripeness of strawberries and identifying ripeness stages suitable for nectar production. However, it is not a tool that can be used to define which strawberries could be used to produce colour stable nectars and it did not give any insights that could not have been deduced from visual inspection of surface colour.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in TU Wien Research Data at https://doi.org/10.48436/jgvdr-01v58.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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