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Prediction of the potential colour stability of strawberry nectar by use of a Stability Prediction Value (SPV)

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ABSTRACT

The colour stabilities of 169 different samples of strawberry nectars produced on a lab-scale were measured over 12 weeks. To find a prediction for consumer acceptance at set times, and therefore a shelf-life estimation, a Stability Prediction Value (SPV) was calculated using colour components L^*, a^* and b^* on day of production. The SPV is the first easily measurable calculated value that can be used to predict the Acceptance Factor (AF) – a measure of how likely a strawberry nectar's colour is accepted by consumers – after 12 weeks. It also can predict how many days have elapsed for a nectar's colour to become unacceptable, giving a shelf-life prediction. The SPV values were also calculated for 21 samples produced industrially, and SPV was found to also predict AF after 12 and 36 weeks. SPV, for the first time, gives a quick and simple method to screen raw materials for nectar production, and so could be used by producers to make more colour stable nectars, and breeders to guide development of cultivars. Additionally the potential of the colour component L*, and the concentration of anthocyanins to predict colour stability was evaluated.

1. Introduction

The colour instability of nectars made of strawberries (*Fragaria x ananassa*) poses a difficult problem for the producers and retailers of these nectars. Strawberry nectars straight after production will be a bright red colour, attractive to consumers. Unfortunately this colour is not stable and will soon (in a matter of weeks) degrade to an unattractive brown colour, unacceptable to consumers. (Gössinger et al., 2009 (a)). This is particularly problematic for nectar production as strawberry nectars can, by EU directive, only contain strawberry, water, citric acid and sugar (Anon, 2014). Therefore nectar producers cannot legally add any of the extra colourants that are commonly added to other products (such as Strawberry Juice Drinks) to hinder colour degradation. If a product's colour becomes unacceptable to consumers, it will no longer be sellable. The likelihood that a nectar will be accepted by a consumer can be quantified by use of the Acceptance Factor (AF) (Gössinger et al., 2009 (a)). It is calculated using the CIELAB colour components: a^* (green-red), b^* (blue-yellow) L^* (lightness), from which C^* (Chroma) and h (hue angle) are calculated. (Kammerer, Schillmöller, Maier, Schieber, & Carle, 2007). The AF is derived as follows: $AF = \frac{a^*}{h^\circ}$. Nectars with AF > 0.7 were considered excellent and those with AF < 0.4 were considered unacceptable. (Gössinger et al., 2009 (a)). A measure of shelf life can be determined by measuring in days how long a nectar takes to reach an AF where it is no longer acceptable. When a nectar's poor colour means it can no longer be sold, this can cause financial losses to producers and retailers, and also contribute to food wastage. For this reason it is advantageous to produce nectars which are as colour stable as possible. There is currently no simple test to indicate the potential colour stability of nectars. A prediction of AF after a set time would be advantageous as it gives a real indication of how acceptable a sample is to consumers over time. Knowing the potential colour stability of a nectar would be very useful as it could be used to dictate factors such as stock rotations, and pricings of nectars (with those with greater stability demanding higher prices). Additionally this would help breeders screen cultivars and guide breeding to quickly find those cultivars that are the most colour stable. Therefore a simple test to predict how long a nectar would be colour stable would be of great interest to industry.

There have been many previous studies into the factors that affect colour stability. The degradation of the colour is driven by the degradation of the anthocyanin pigments, which give the strawberries their initial bright colour. The two most significant of which are pelargonidin

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Abbreviations

AF	Acceptance Factor
AF0	Acceptance Factor on Day of Production
AF4	Acceptance Factor after 4 weeks
AF8	Acceptance Factor after 8 weeks
AF12	Acceptance Factor after 12 weeks
AF24	Acceptance Factor after 24 weeks
AF36	Acceptance Factor after 36 weeks
D04	Difference in Acceptance Factor between week 0 and week 4
D08	Difference in Acceptance Factor between week 0 and week 8
D12	Difference in Acceptance Factor between week 0 and week 12
D24	Difference in Acceptance Factor between week 0 and week 24
D36	Difference in Acceptance Factor between week 0 and week 36
L0	Lightness value L^* on day of production
a0	Redness a^* value on day of production
b0	Yellowness b^* value on day of production
C0	Chroma C^* value on day of production
h0	Hue angle h^* value on day of production
SPV	Stability Prediction Value

3-glucoside and cyanindin 3-glucoside. (Bakker, Bridle, & Bellworthy, 1994; Garzon & Wrolstad, 2002; Gimenez, Kajda, Margomenou, Piggott, & Zabetakis, 2001). Previous studies have found many different factors that affect the degradation of the colour, including temperature, L-ascorbic acid content, pH value, enzyme activities and non-enzymatic browning (Wrolstad, Putnam, & Varseveld, 1970; Garcia-Viguera et al., 1999; Gössinger et al., 2009 (a); Gössinger et al., 2014). It is currently controversial whether the initial concentration of anthocyanins have significant impact on colour stability. Some studies have found that a higher concentration is an indicator of stability (Diamanti et al., 2015; Skrede, Wrolstad, Lea, & Enersen, 1992), and anthocyanin concentration was suggested, alongside other factors (a^* value, total colour change (ΔE^*), total anthocyanin and L-ascorbic acid content) as an analytical marker for shelf-life predictions (Buvé et al., 2018), with concentration cut off values found for when nectars were past the shelf-life. Alternatively other studies have found that the profile of anthocyanins has a bigger effect on degradation than the total content (Garzon & Wrolstad, 2002; Teribia et al., 2021), additionally other studies found that the concentration of anthocyanins doesn't have a significant impact on the colour stability of nectars (Gössinger et al., 2009 (c)), and that other factors, such as the ripening stage, are important to the stabilities despite having lower anthocyanin concentrations (Gössinger et al., 2010). A disadvantage of anthocyanin testing is that it is a very involved process, which must be undertaken with specialist equipment by trained professionals, making it unsuitable as a predictive quality parameter in industrial settings.

While the processing of nectars, such as freezing or pasteurisation, can have an effect on the colour stability of the nectars (Gössinger et al., 2009 (b)), the raw material is a very important factor in the colour stability. It has been shown that some cultivars are inherently more stable than others, and those strawberries that are more ripe and from a later harvest point are more stable (Gössinger et al., 2014). Based on this knowledge that the raw material of strawberry nectars can be so diverse, this study aimed to screen a large number of distinct strawberry samples for colour stability. Indicators of colour stability were compared to parameter measurements taken on day of production to see if any could be used as a predictive tool for colour stability. Additionally a smaller range of samples were analysed for anthocyanin content, to better understand if the concentration of anthocyanins can be used to predict colour stability. Samples produced on an industrial scale were also screened for colour stability to validate that findings from the lab-scale could be applied to an industrial scale.

2. Material and methods

2.1. Strawberries

2.1.1. Strawberries from Germany

In the harvest season 2020, 118 different cultivars of strawberries (*Fragaria x ananassa),* grown in Dresden (Germany), were harvested fully ripe for processing (riper than those destined for retail) in the morning and frozen at -18 °C. After the harvest period, these fruits were defrosted overnight at 4 °C and processed into puree (1-3 kg), returned to -18 °C and transported to Klosterneuburg.

2.1.2. Strawberries from Austria

In June and July 2021 51 samples of strawberries were harvested in from two sites in Lower Austria. These were 14 different cultivars, collected at different ripeness and harvest points. These fruits were picked in the morning and frozen in 5 kg plastic bags at -18 °C within 2 h.

2.2. Strawberry nectar

21 samples of strawberry nectar were supplied by an industrial producer of strawberry nectar. The nectars were produced in Austria from fresh strawberries in June–September 2021. These nectars were produced by the standard process for retail samples of strawberry nectar with homogenisation and degassing. The nectars were bottled in clear 0.75L (18 samples) and 0.25L (three samples) bottles. The samples were stored at 20 °C ± 3 °C in the dark.

2.3. Methods

2.3.1. Preparation of nectars

2.3.1.1. Defrosting of puree. The frozen purees from Germany were defrosted for 24h at 4 °C; samples that were not fully thawed after 24h were brought to 4 °C in a water-bath before analysis and further processing.

2.3.1.2. Defrosting of strawberries and puree preparation. Frozen strawberries from Austria were defrosted for 24h at 20 °C, and then processed into puree by a rotor mill with 1 mm sieve (Feuma, Gößnitz, Germany).

2.3.1.3. Nectar preparation. Purees were mixed with water, citric acid and sugar to produce nectar (40% puree, 15 °Brix, 7.0 g/L Titratable Acidity) and homogenised with a hand blender (Philips, Drachten, Netherlands). 140g ± 1g of nectar was weighed into glass jars (212 ml), sealed with a screw lid, and pasteurised (80 °C 10 min) in a water bath (Westfalia, Hagen, Germany). Nectars were stored at 20 °C ± 3 °C in the dark.

2.3.2. Physical and chemical analysis

Soluble solids (°Brix) was measured with a hand-held refractometer (N-20, Brix 0–20%, ATAGO, Tokyo, Japan). pH-values were determined by use of a pH meter (MultilineP4, WTW, Weilheim, Germany) and pH-electrode (SenTix 41–3, WTW, Weilheim, Germany). Titratable Acidity (TA) was determined by titration to an endpoint of pH-value 7.0 (0.1N NaOH Titrisol ®, Merck KGaA, Darmstadt, Germany) and multiplied by the acidity factor of tartaric acid (0.75) to express acidity as g/L.

2.3.3. Colour measurements

CIELAB-system colour components L^* (lightness), a^* (red-green), and b^* (yellow-blue) were measured by a Minolta 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 (Kammerer et al., 2007). AF was calculated as previously reported (Gössinger et al., 2009 (a)) All nectars were measured after pasteurisation on day of production. The samples from Germany were tested weekly, but only until they were below AF 0.4. Samples from Austria were measured weekly until the AF was below 0.4, but in order to gain statistical data they were tested at 4, 8, and 12 weeks even when below AF 0.4. Industrial samples were tested every 4 weeks for 36 weeks. Each sample was measured twice, in duplicate. The colour stability was quantified with different indictors of colour stability: AF04, AF08, AF12 is the AF at 4, 8 and 12 weeks. D04, D08 and D12 are the difference in AF between the day of production and 4, 8 and 12 weeks respectively. Also quantified was Days to AF 0.5 — a point where samples were unacceptable to some, although not all, consumers. This was measurement was chosen as it included all samples with AF > 0.5 on day of production (167 samples).

2.3.4. Anthocyanin determination

Anthocyanins were determined from nectar by HPLC by a modification of the method described in (Eder, Wendelin, & Barna, 1990), as described in (Gössinger et al., 2022). Analysis was performed on samples of pasteurised nectar frozen on day of production, and stored at -18 °C. Total anthocyanin concentration was expressed as pelargonidin 3-glucoside [mg/L].

2.3.5. Statistical analysis

Statistical analysis (Analysis of variance, Tukey-HSD, Curve fitting, Regression Analysis, and Pearson Correlations) was carried out using IBM SPSS 26 (Statistical Package for the Social Sciences).Trend line analysis was made using Microsoft Excel.

3. Results and discussion

3.1. Grouping of samples by stability

After 12 weeks the lab-scale samples were grouped according to their AFs, to give 6 distinct stability groups, shown in Table 1. Grouping was necessary because the samples from Germany were no longer tested once they reached AF < 0.4. This is due to this study initially only looking at the effect of the cultivar on colour stability. In consequence, of the 169 initial samples there were only 165 with measurements after 4 weeks, 139 after 8 weeks, and 111 samples with measurements for the full 12 weeks, with the data skewed towards more stable samples. The groupings allowed statistical comparison of very unstable samples with those more stable, which would otherwise not have been possible.

Table 1
Showing the number of samples in each stability grouping, defined by the Acceptance Factor of the nectars during storage. Also shown are the values for °Brix, Titratable Acid (TA), for all the purees processed to nectar at lab-scale, and pH-value for lab-scale nectars by stability grouping. Different lower case letters (vertical) illustrate significant differences ($p < 0.05$).

Stability group number	Description	Number of Samples	°Brix	Titratable Acid [g/L]	pH-value
1	AF <0.4 after 4 weeks	9	9.9 ± 0.6 b	10.9 ± 1.9 b	3.7 ± 0.1 a
2	AF <0.4 after 8 weeks	38	8.9 ± 1.1 a	9.7 ± 1.8 a	3.6 ± 0.1 a
3	AF <0.4 after 12 weeks	35	9.2 ± 1.2 a	9.1 ± 2.1 a	3.5 ± 0.5 a
4	AF 0.4–0.5 after 12 weeks	41	9.1 ± 1.0 a	9.7 ± 2.2 a	3.6 ± 0.5 a
5	AF 0.5–0.6 after 12 weeks	32	9.1 ± 1.0 a	9.5 ± 2.1 a	3.7 ± 0.3 a
6	AF >0.6 after 12 weeks	14	8.8 ± 0.8 a	9.9 ± 2.4 a	3.6 ± 0.3 a

3.2. Brix, TA and pH-value

The means of Brix and TA of the puree, and the pH-values of the nectars, produced at lab-scale, for each grouping, are shown in Table 1. For both the Brix and the TA the only significant difference was between the least stable group (1) and all other groupings. No significant difference was found between the pH values for the different stability groupings. These are therefore not suitable parameters for stability predictions.

3.3. Colour measurements on day of production

The means of the different colour components of the nectars produced at lab-scale, as measured on the day of production, for each grouping are listed in Table 2. For the values of b^* and the Chroma C^* the only significant difference was between the most unstable group and all the other samples. The values of a^* showed no significant differences between stable groups 3-6, but significant difference between these and the unstable groups 1 & 2. AFs are proportional to the a^* values, so it follows that those with particularly low a^* values would have lower AF values, and therefore likely to be below AF 0.4 sooner. The initial AF also had no significance between groups 4-6, with significant differences only between the least stable groups. This suggests these colour parameters are not a good parameters to predict colour stability.

3.4. L0 as a predictor of colour stability in lab-scale samples

The initial value of L^* (L0) had statistical significance between all groups except groups 2-3, as seen in Table 2. Those with the worst stabilities (AF < 0.4 before 4 weeks) had an average L0 value of 33.28 ±

Table 2
Showing the colour component values a*, b* C*, h°, L* and Acceptance Factor for each stability group on the day of nectar production (a0, b0, C0, h0, L0 and AF0), and the Stability Prediction Value (SPV), for all the nectars produced at lab-scale, by Stability Grouping. Different lower case letters (vertical) illustrate significant differences ($p < 0.05$).

Stability Group Number	a0	b0	C0	h0	L0	AF0	SPV
1	24.47 ± 4.25 a	17.99 ± 2.09 a	30.40 ± 4.49 a	36.62 ± 3.01 d	33.28 ± 3.16 a	0.68 ± 0.15 a	0.82 ± 0.37 a
2	28.97 ± 2.03 b	20.49 ± 1.91 b	35.49 ± 2.64 b	35.23 ± 1.43 c	25.26 ± 2.93 b	0.82 ± 0.06 b	1.91 ± 0.36 b
3	29.68 ± 2.23 bc	20.50 ± 2.04 b	36.08 ± 2.91 b	34.58 ± 1.27 bc	23.07 ± 2.79 b	0.86 ± 0.06 c	2.48 ± 0.51 c
4	30.31 ± 1.60 c	20.68 ± 2.14 b	36.71 ± 2.38 b	34.21 ± 1.84 b	21.81 ± 2.03 c	0.89 ± 0.05 cd	2.92 ± 0.58 d
5	30.21 ± 2.86 c	20.07 ± 2.88 b	36.18 ± 2.42 b	33.47 ± 1.93 a	20.82 ± 2.01 d	0.90 ± 0.07 d	3.22 ± 0.47 e
6	30.27 ± 1.70 c	19.79 ± 2.01 b	35.88 ± 2.42 b	33.10 ± 1.71 a	17.89 ± 1.41 e	0.92 ± 0.05 d	4.46 ± 0.67 f

3.16, this compares with the most stable group (AF > 0.6 after 12 weeks) having an L0 value of 17.89 ± 1.41. This suggested that L0 could be used as a predictor of colour stability. Simple linear regression was used to test if L0 significantly predicted D12. As seen in Fig. 1 the fitted regression model was:

$$D12 = 0.024*L0 - 0.085 \tag{1}$$

The overall regression was statistically significant ($R^2 = 0.462$, $F(1, 534) = 459.352$, $p < 0.001$). It was found that L0 significantly predicted D12 ($\beta = 0.680$, $p < 0.001$). The regression coefficient ($B = 0.024$, 95% CI [0.021, 0.026]) indicated that increasing L0 by 1 increased D12 by 0.024. As shown in Fig. 1 the regression coefficient was even better for D04 ($R^2 = 0.593$), this is probably due to the fact that those with very high L0 values had very large drops initially, and then didn't degrade much further. Fig. 2 shows L0 was a less useful tool for predicting the AF after 12 weeks ($R^2 = 0.294$). The correlation coefficient of L0 Days to AF 0.5 is -0.580 ($p < 0.01$). The relationship between Days to AF 0.5 and L0 was non-linear, therefore the natural log of the Days to 0.5 was taken to allow linear regression, shown in Fig. 3. The fitted regression model was:

$$\ln(\text{Days to } 0.5) = 6.926 - 0.124*L0 \tag{2}$$

The overall regression was statistically significant ($R^2 = 0.541$, $F(1, 770) = 907.453$, $p < 0.001$). It was found that L0 significantly predicted the natural log of Days to AF 0.5 ($\beta = 0.736$, $p < 0.001$). The regression coefficient ($B = -0.124$, 95% CI [-0.132, -0.116]) indicated each unit increase in L0 the number of Days to AF 0.5 decreases by 11.7%. While this can give a shelf-life prediction, the lower correlation coefficient means that rather than shelf-life, L0 is better as predictor of the degradation of AF over time. D12 can be misleading, however, as samples with poor initial colour (AF <0.5) can have small differences because they started with very low AF values initially. Similarly samples with a good colour initially can have a large difference, but still be acceptable after this time. A different measurement is needed to give a better prediction for the AF values at set times, and improve the shelf-life prediction.

3.5. Prediction Tool and stability prediction value

With the knowledge that L0 is linked to the colour stability, it was used as the basis for the creation of a mathematical Prediction Tool (Equation (3)), which could be used to predict AF after a set time, only using colour measurements (a^* and b^*) from day of production (a0 and b0).

$$\frac{a0^3}{L0^2 * b0} = \textit{Stability Prediction Value} \tag{3}$$

The result of Equation (3) is the Stability Prediction Value (SPV). The mean SPV for the different stability groupings can be seen in Table 2. Significant differences were found between all of the groups, and these were sequential, with the lowest stability groups having the lowest SPVs (Group 1: 0.82 ± 0.37), and the highest stability groups having the highest SPVs (Group 6: 4.46 ± 0.67). Fig. 5 shows the AF against the SPV at 0, 4, 8 and 12 weeks. The regression equation of SPV with AF12 is:

$$AF12 = 0.116*SPV + 0.103 \tag{4}$$

The overall regression was statistically significant ($R^2 = 0.586$, $F(1, 534) = 754.87$, $p < 0.001$). It was found that SPV significantly predicted AF12 ($\beta = 0.765$, $p < 0.001$). The regression coefficient ($B = 0.116$, 95% CI [0.108, 0.124]) indicated that increasing SPV by 1 increased AF12 by 0.116. The SPV is therefore a good predictive tool for predicting an AF after a set time. Fig. 5 shows that the R^2 and R values decreased slightly over time. This is probably due to not having data for the very unstable German samples at these time points, this skewed the data towards the stable samples at later time points. The correlation coefficient of SPV

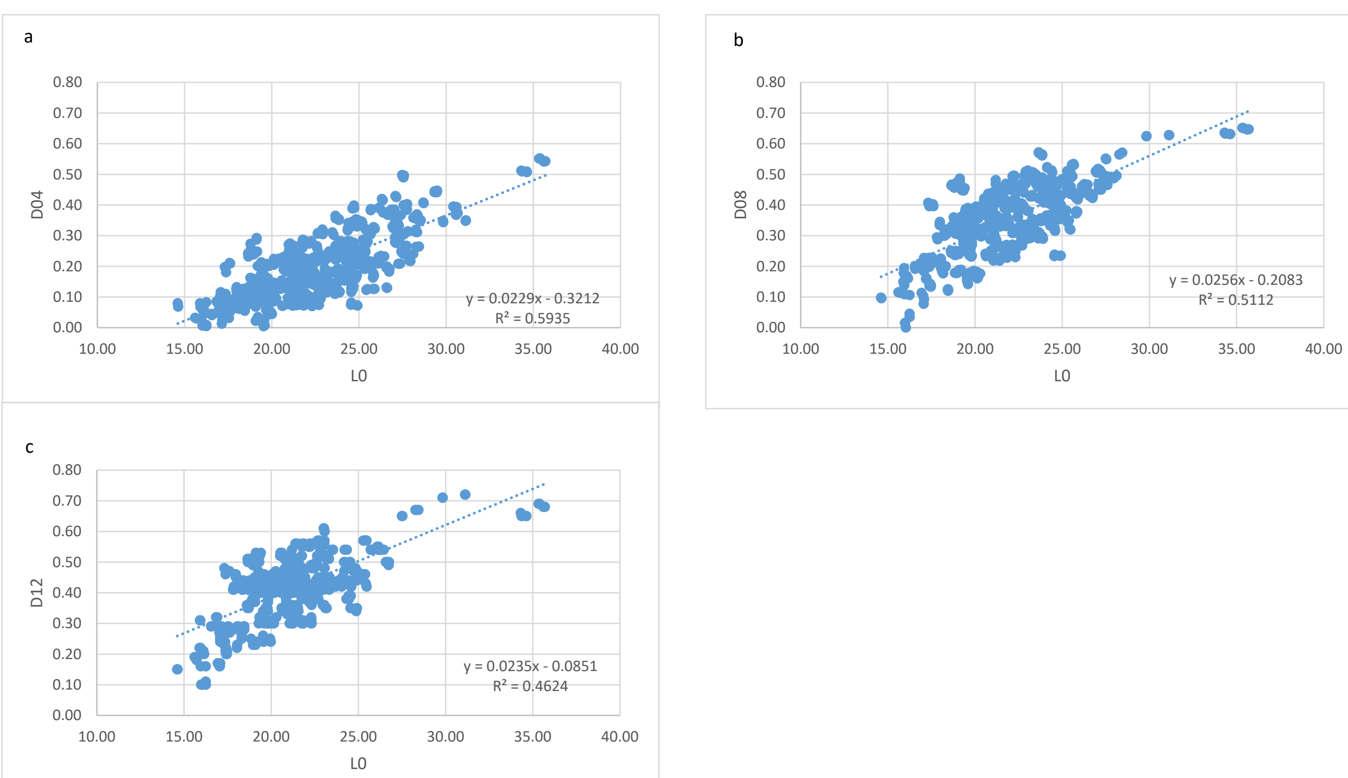

Fig. 1. Difference in Acceptance Factor between day of production and after 4 (a), 8 (b) and 12 (c) weeks plotted against Initial Lightness L* Value (L0) for samples made at lab-scale. All regression coefficients were found to have $p < 0.001$. Person Correlation coefficients were found to be (a) 0.714, (b) 0.715 and (c) 0.699 (all $p < 0.01$).

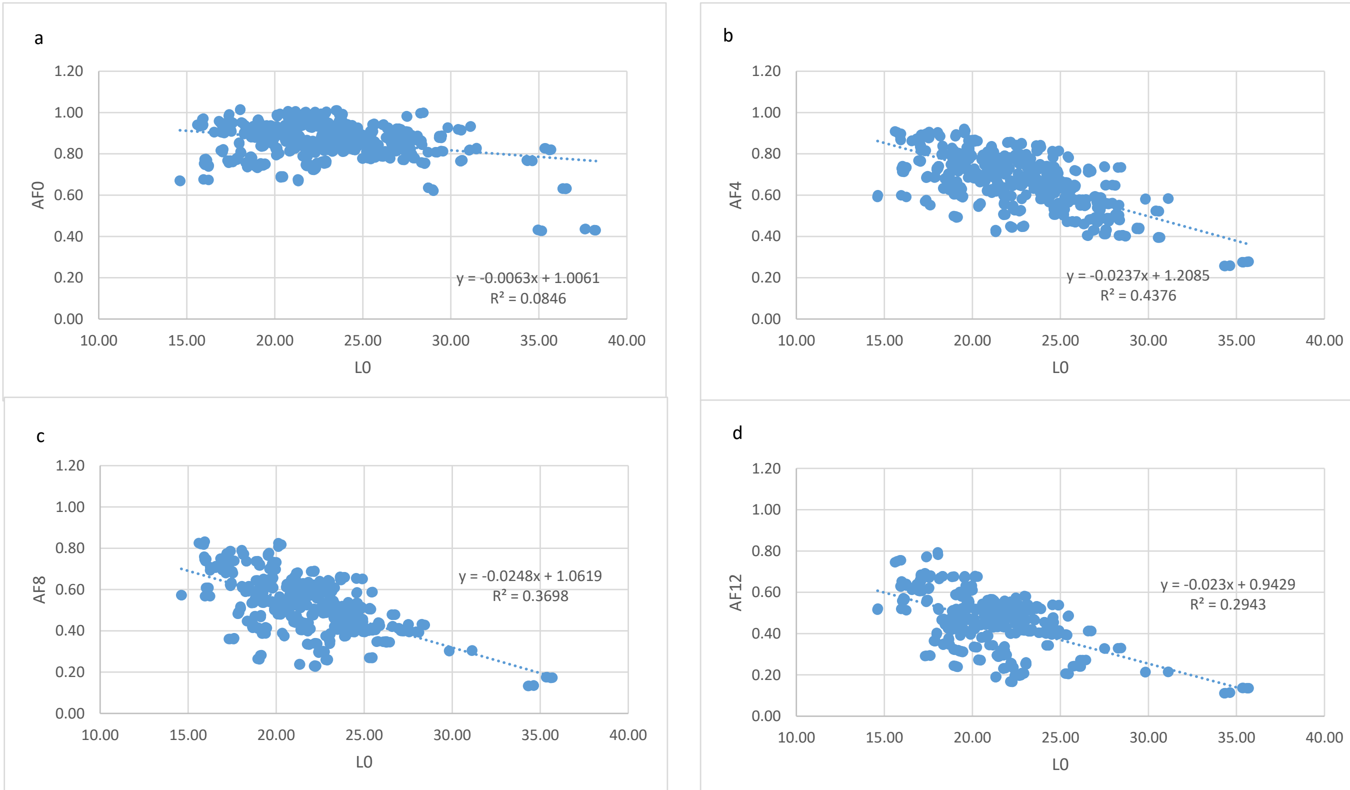

Fig. 2. Acceptance Factor after 0 (a), 4 (b), 8 (c) and 12 (d) weeks plotted against Initial Lightness L* Value (L0) for samples produced at lab-scale. All regression coefficients had $p < 0.001$. Pearson Correlation coefficients were (a) -0.291, (b) -0.661, (c) -0.608 and (d) -0.542 (all $p > 0.01$).

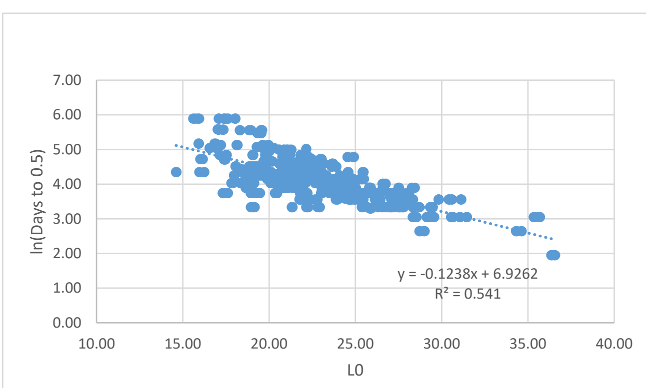

Fig. 3. Natural log of the number of days taken for Acceptance Factor to fall below 0.5, at initial values of L* (L0) for the lab-scale samples. All regression coefficients had $p < 0.001$.

with Days to 0.5 was better than that for L0 at 0.764 ($p < 0.01$). Again, the relationship was non-linear and so the natural logs of both parameters allowed linear regression. As shown in Fig. 6 the fitted regression model was:

$$\ln(\text{Days to } 0.5) = 1.44^{*}\ln(\text{SPV}) + 2.76 \quad (5)$$

The overall regression was statistically significant ($R^2 = 0.726$, $F(1, 770) = 2036.6$, $p < 0.001$). It was found that the natural log of the SPV significantly predicted the natural log of Days to AF 0.5 ($\beta = 0.852$, $p < 0.001$). The regression coefficient (B = 1.44, 95% CI [1.380, 1.506]) indicated a 10% increase in SPV increases the number of Days to AF 0.5 by 14.7%. The difference in correlation between the natural log and the original data shows this model is likely not perfect. This is likely due to the bias towards the few very unstable samples, although this does help easily identify those samples that were very unstable, and therefore particularly unsuitable for nectar production. Despite this SPV is a good tool for predicting both the AF after 12 weeks, and the Days to AF 0.5. Fig. 4 showed SPV was less suitable for predicting D12 than L0, demonstrating the two different parameters are suitable for predicting different indicators of colour stability.

3.6. Industrial samples

To validate if the SPV would also be effective at predicting the stability of industrially produced samples the SPV was calculated for 21 samples that had been produced on an industrial scale. Before processing the purees had average Brix values of 8.64 ± 1.15°Bx, TA of 7.97 ± 2.14 g/L and pH-value of 3.52 ± 0.16. The correlation coefficients between these values and the AF36 were -0.432, -0.094, and -0.194, respectively ($p > 0.01$). These samples were much more stable than those produced at lab scale and they all remained above AF 0.4 for more than 36 weeks. Like the lab samples the L0 value was a good predictor of the difference in AFs between production and 12, 24 and 36 weeks, as shown in Table 3, but was not as good a predictor of the AF at these times. This again shows that while L0 is a good predictor of overall stability it is not a good parameter to predict whether a sample would be acceptable to consumers after a set time. As shown in Fig. 7 the SPV was a good predictor of the AFs after 12, 24 and 36 weeks. However, the regression equation for AF12 was very different to that the lab-scale study (Equation (4)):

$$AF12 = 0.217^{*}SPV + 0.296 \quad (6)$$

The overall regression was statistically significant ($R^2 = 0.57$, $F(1, 82) = 108.790$, $p < 0.001$). It was found that SPV significantly predicted AF12 ($\beta = 0.755$, $p < 0.001$). The regression coefficient (B = 0.217, 95% CI [0.175, 0.258]) indicated that increasing SPV by 1 increased AF12 by

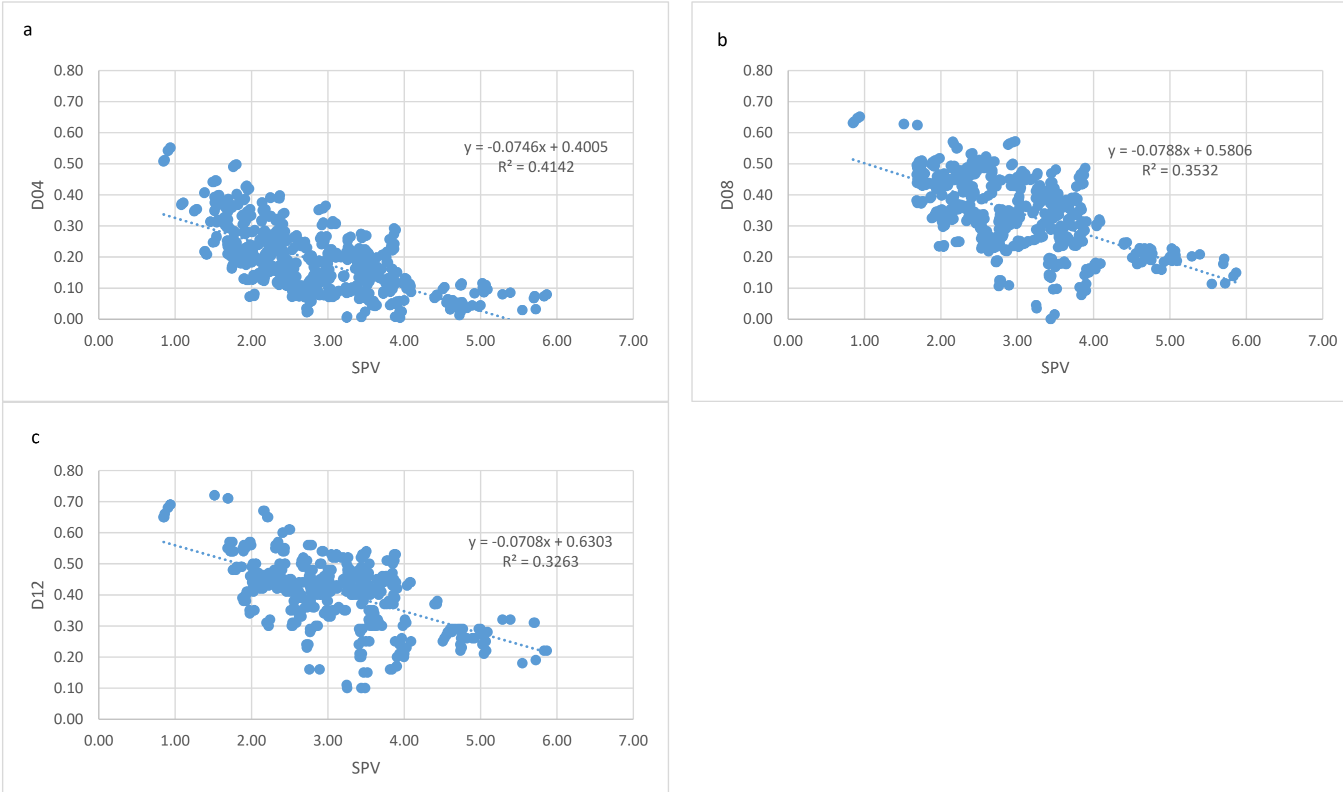

Fig. 4. Difference in Acceptance Factor between day of production and after 4 (a), 8 (b) and 12 (c) weeks plotted against Stability Prediction Value (SPV) for the samples made at Lab-scale. All regression coefficients were found to have $p < 0.001$. Pearson Correlation coefficients were (a) -0.644, (b) -0.571 and (c) -0.571 (all $p > 0.01$).

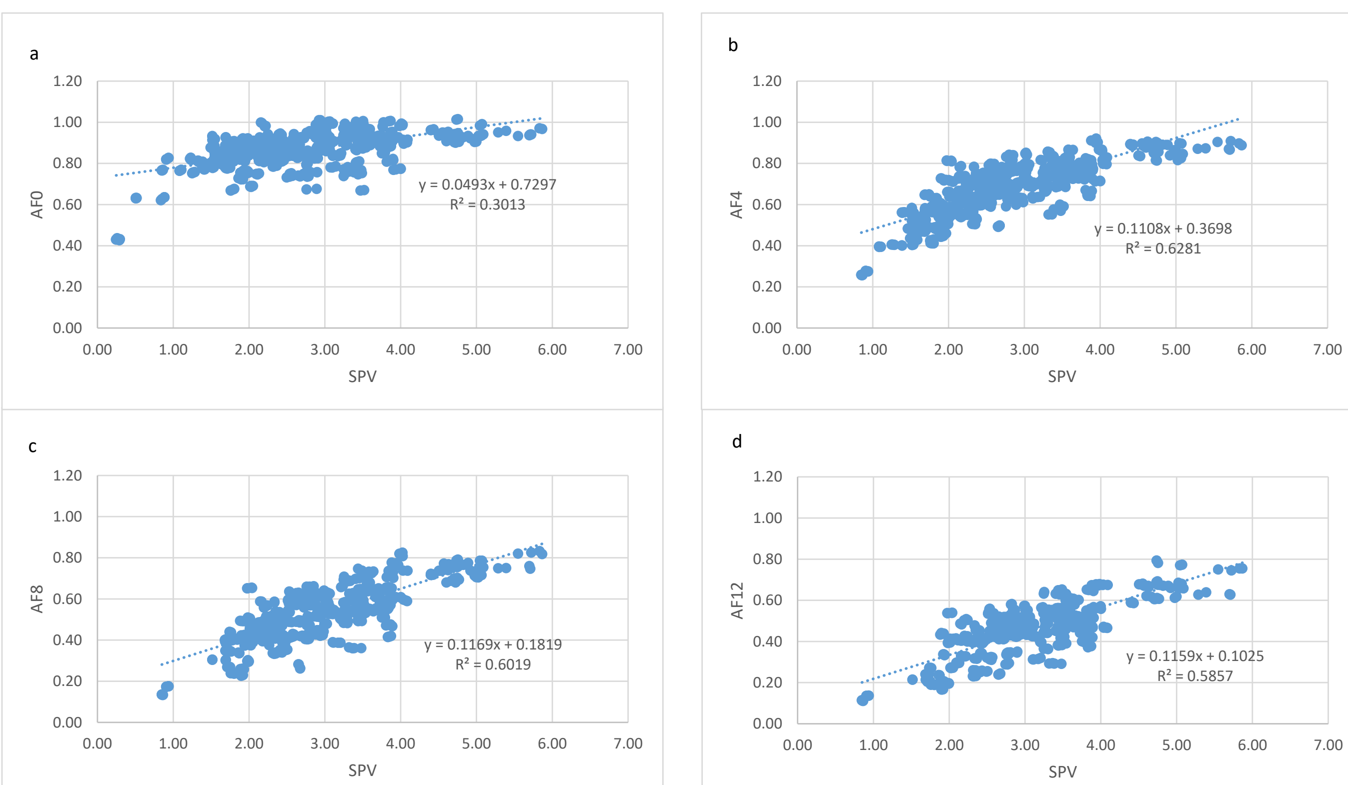

Fig. 5. Acceptance Factor after 0, (a), 4 (b), 8 (c) and 12 weeks, plotted against Stability Prediction Value (SPV) for the samples produced at lab-scale. All regression coefficients had $p < 0.001$ Pearson correlation coefficients were (a) 0.549, (b) 0.793, (c) 0.776 and (d) 0.765 (all $p > 0.01$).

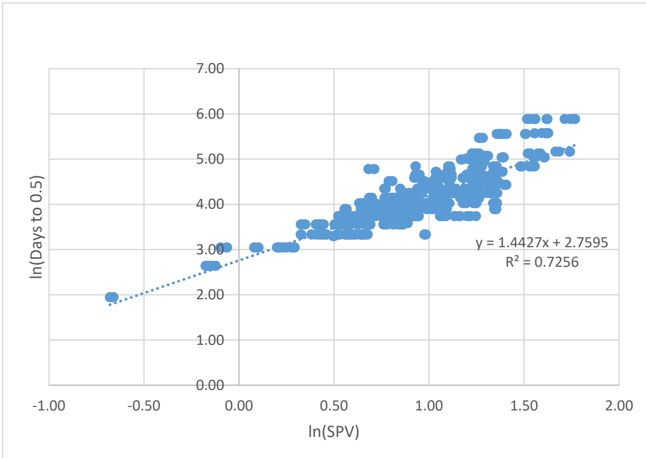

Fig. 6. Natural Log of Number of days taken for Acceptance Factor to fall below 0.5 at the natural log of different Stability Prediction Values (SPV) for the lab-based samples. All regression coefficients had $p < 0.001$.

0.217 – nearly double the value for the lab-scale samples. While the data shows that the SPV is a good predictor for the AF after 12 weeks, with similar correlation and regression values to the lab-scale counterpart, the values of SPV are not interchangeable between these studies. This is possibly because lab-based samples lack the homogenisation and degassing steps present in normal industrial production, and have a larger headspace meaning they are more oxygenated than those produced industrially. Greater oxidation leads to faster colour degradation (Howard, Brownmiller, & Prior, 2014), and so these lab samples could be seen as a form of accelerated shelf-life testing when compared to normal industrial production. Due to only one sample being below AF 0.5 after 36 weeks it was not possible to generate an equation for the shelf-life of these industrial samples. Due to the differences in the absolute SPV values the shelf-life of the industrial samples couldn't be estimated by using the regression equations for the lab samples. This disparity means that while the SPV is a useful tool to compare and rank those samples that have all been produced in the same way, the absolute values cannot be used to compare nectars made by different methods. Despite this SPV is still of use as a comparison tool – fruit processors and bottlers can demand higher prices for those nectars that are more stable and SPV can be used to screen raw materials to find which will be the most stable, and set pricing accordingly. It would also be of use to breeders who could use it to quickly screen potential colour stability of strawberry cultivars, to guide future breeding.

3.7. Anthocyanins

From the lab-scale samples analysed for colour stability a selection of 37 samples were taken for anthocyanin determination. These were a selection of the most and least stable, as well as those cultivars that are of industrial interest. The number from each stability grouping can be seen in Table 4 alongside the concentrations of cyanidin-3-glucoside, pelargonidin-3-glucoside and the total concentration of anthocyanins for each of the stability groups. There was no difference between the groupings and the concentration of cyanidin-3-glucoside, but both the concentration of pelargonidin-3-glucoside and the total anthocyanins showed that the later groups had significant differences, with higher concentrations in the most stable groupings. The regression analysis of the total concentration of anthocyanins with colour stability indicators can be seen in Table 5. The anthocyanin concentration was fairly good at predicting AF12 ($R^2 = 0.439$), but this was lower than that of the SPV. It was not a good predictor of D12 ($R^2 = 0.225$), showing that L0 is a better parameter to predict difference in colour stability. The correlation coefficient of anthocyanin concentration with Days to AF 0.5 was 0.627 ($p < 0.01$), a less good correlation than that of SPV. As with the SPV, the natural logs were taken so that simple linear regression could be used to test if the concentration of total anthocyanins significantly predicted the Days to AF 0.5 (this figure was available for all 37 samples). As shown in Fig. 8, the fitted regression model was:

$$\ln(\text{Days to } 0.5) = 1.10 * \ln(\text{Anthocyanin Conc.}) - 1.01 \tag{7}$$

The overall regression was statistically significant ($R^2 = 0.527$, $F(1, 174) = 193.92$, $p < 0.001$). It was found that the natural log of the concentration of anthocyanins significantly predicted the natural log of Days to AF 0.5 ($\beta = 0.726$, $p < 0.001$). The regression coefficient ($B = 1.101$, 95% CI [0.945, 1.257]) indicated each 10% increase in anthocyanin concentration increases the number of Days to AF 0.5 by 11.1%. While this shows that anthocyanin concentration can be used as a predictor of the shelf life, it is a worse prediction than that of the SPV. Also these had far fewer samples than the model for the SPV; future study would be necessary to find if these results would be the same with a larger sample size, comparative to that of the SPV. This would suggest, along with the lack of consensus in the literature, and the fact that anthocyanin testing is an involved process requiring professional knowledge and equipment, that while anthocyanins concentration is a predictor of colour stability of nectars, SPV is a better tool for predicting colour stability of strawberry nectars in industrial settings.

4. Conclusions

The colour component L^* on day of production (L0) is a predictor of absolute colour stability, with those nectars with low L^* values having higher stability - smaller changes in customer acceptability over time. Using L0, alongside other colour values obtained on the day of production, a Stability Prediction Value was calculated. The SPV is a measurement that can be obtained using a quick and simple colorimetry method, on the day of production, to predict the consumer acceptance over time. The SPV predicted both the consumer acceptability after 12 weeks, and the number of days for a sample to fall below a set acceptability level. While the SPV predicted colour stabilities for both lab-scale and industrial samples, these had very different regression equations. This meant that the SPVs of the two studies couldn't be compared, and therefore absolute values of SPV can't be used to compare nectars produced by different methods. The lack of comparability would suggest that the SPV only gives a measurement of the potential innate stability of

Table 3
Regression Table of Lightness L* on day of production (L0) of the industrial samples (21 samples) with indicators of colour stability – Acceptance Factor at 0,12,24 and 36 weeks (AF0, AF12, AF24 and AF36), difference in acceptance factor between week 0-12, week 0-24 and week 0-36 (D12, D24 and D36).Standard error reported in brackets. Significance: *p < 0.05 **p < 0.01 ***p < 0.001.

Predictive Parameter	AF0	AF12	AF24	AF36	D12	D24	D36
Constant	0.462***	1.119***	1.176***	1.132***	-0.658***	-0.715***	-0.671***
	(0.094)	(0.131)	(0.145)	(0.151)	(0.090)	(0.106)	(0.115)
L0	0.021	-0.011*	-0.016**	-0.018**	0.032***	0.038***	0.039***
	(0.004)	(0.005)	(0.006)	(0.006)	(0.003)	(0.004)	(0.004)
R	0.545	0.229	0.312	0.322	0.714	0.715	0.699
R-Squared	0.297	0.052	0.097	0.104	0.510	0.511	0.489

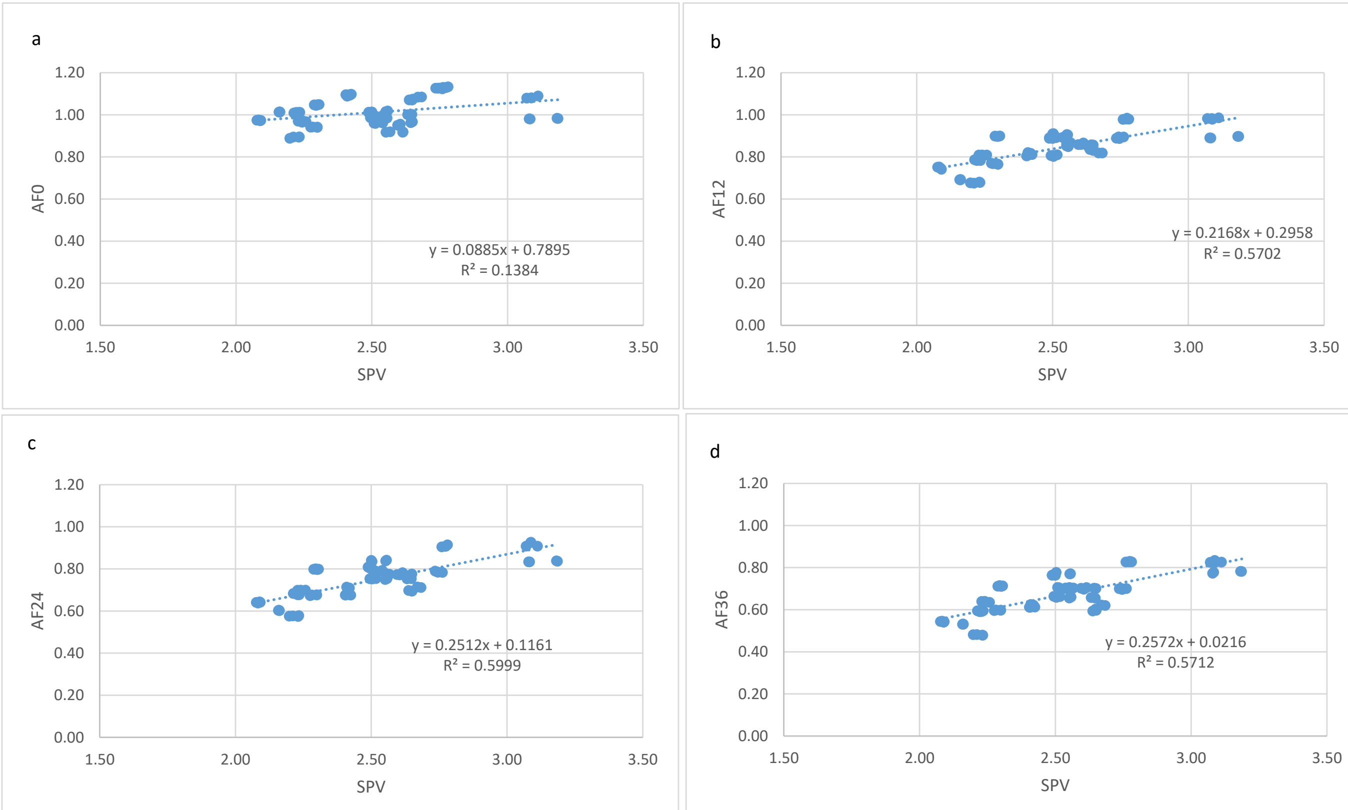

Fig. 7. Acceptance Factor after 0, (a), 12 (b), 24 (c) and 36 weeks, plotted against Stability Prediction Value (SPV) for the samples produced at industrial scale. For (a) and (b) all regression coefficients had $p < 0.001$. For (c) and (d) regression coefficients for the SPV had $p < 0.001$. For (c) the regression coefficient for the constant had $p = 0.046$ and (d) $p = 0.729$ Pearson correlation coefficients were (a) 0.372, (b) 0.755, (c) 0.755 and (d) 0.756 (all $p < 0.01$).

Table 4
Showing the number of samples in each stability grouping analysed for anthocyanin content, defined by the Acceptance Factor of the nectars during storage. Also shown are the concentration of Cyanidin-3-glucoside [mg/L], Pelargonidin-3-glucoside [mg/L] and the total concentration of Anthocyanins expressed as Pelargonidin 3-glucoside [mg/l] for the lab-based samples taken for anthocyanin testing. Different lower case letters (vertical) illustrate significant differences ($p < 0.05$).

Stability group number	Number of samples	Cyanidin-3-glucoside [mg/L]	Pelargonidin-3-glucoside [mg/L]	Total Anthocyanins [mg/L]
1	5	2.56 ± 0.49 ab	51.04 ± 37.96 a	71.59 ± 42.64 a
2	7	1.74 ± 1.05 a	67.43 ± 15.82 ab	91.44 ± 26.96 ab
3	2	3.13 ± 2.44 ab	78.79 ± 46.22 ab	102.07 ± 58.35 ab
4	4	1.61 ± 0.30 a	90.18 ± 20.50 b	136.16 ± 34.00 bc
5	8	3.10 ± 1.47 ab	126.73 ± 57.86 c	168.88 ± 86.38 c
6	11	3.72 ± 2.88 b	178.54 ± 51.96 d	230.15 ± 68.72 d

raw material, not the overall stability of a nectar. These findings coheres with previous study, which found that different processing methods, such as pasteurisation and pre-freezing regimes can help stabilise nectars (Gössinger et al., 2009 (b); Gössinger et al., 2009 (c)) and it is unknown what difference in SPV would be observed between samples produced by different processes. While this means that SPV cannot give a shelf-life for any nectar made by any process, it can still be used to compare nectars made in the same way. SPV would therefore still be of use in industry, as it can be used to quickly screen raw materials, to find which raw material would give the most stable nectars. Using the SPV producers could easily find which strawberries would be best to use, and growers could set higher prices accordingly. The SPV would also be of use to breeders, as they could use the SPV to guide breeding by quickly being able to screen cultivars, to find those that have the best potential to be colour stable and therefore focus their research. Further research could be used to link together the SPV values found in the lab-based trial, and the industrial scale by producing nectars out of the same raw materials in both methods, to potentially find a factor to convert the SPV from lab to industrial. In this study it was also found that anthocyanin concentration was correlated to the AF after a set time, and could be used as predictor of shelf-life, but was not as good a predictor as the SPV. In conclusion the Stability Prediction Value was found to be the first parameter known to predict the colour stability and shelf-life of strawberry nectars on the day of production through a quick and simple method.

CRediT authorship contribution statement

Helen Murray: Conceptualization, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Claudia Dietl-Schuller:** Investigation. **Marlene Lindner:** Investigation. **Karin Korntheuer:** Methodology, Investigation. **Heidrun Halbwirth:** Resources, Project administration. **Manfred Gössinger:** Conceptualization, Supervision, Methodology, Writing – review & editing.

Declaration of competing interest

None.

Table 5
Regression Analysis of the total Anthocyanin concentration expressed as Pelargonidin 3-glucoside [mg/L] of selected lab-scale nectars with indicators of colour stability – Acceptance Factor at 0, 4, 8 and 12 weeks (AF0 (37 samples), AF4 (35 samples). AF8 (33 samples) and AF12 (30 samples)), and difference in acceptance factor between week 0-4, week 0-8 and week 0-12 (D04 (35 samples), D08 (33 samples) and D12 (30 samples)). Standard error reported in brackets. Significance: *p < 0.05 **p < 0.01 ***p < 0.001.

Predictive Parameter	AF0	AF4	AF8	AF12	D04	D08	D12
Constant	0.764***	0.479***	0.309***	0.247***	0.288***	0.457***	0.504***
	(0.011)	(0.018)	(0.022)	(0.026)	(0.015)	(0.020)	(0.023)
Total Anthocyanins [mg/L]	0.001***	0.001***	0.002***	0.001***	-0.001***	-0.001***	-0.001***
	(0.000)	(0.000)	(0.001)	(0.000)	(0.000)	(0.000)	(0.000)
R	0.582	0.737	0.710	0.663	0.606	0.573	0.474
R-Squared	0.339	0.543	0.503	0.439	0.367	0.328	0.225

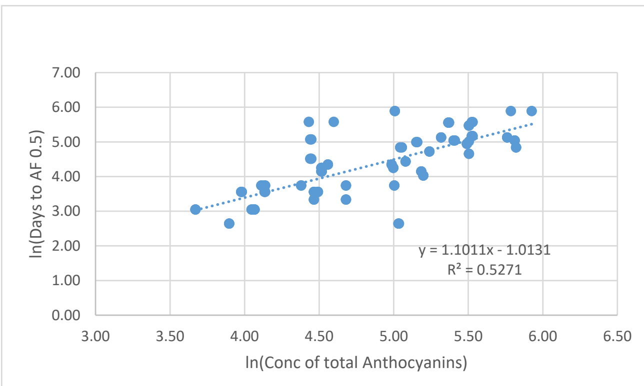

Fig. 8. Natural Log of Number of days taken for Acceptance Factor to fall below 0.5 at the natural log of the concentration of total anthocyanins expressed as Pelargonidin 3-glucoside in [mg/L]. Regression coefficient for ln(concentration) had $p < 0.001$, for the constant $p = 0.01$.

Data availability

The authors do not have permission to share data.

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