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Strawberry Post-Harvest Anthocyanin Development to Improve the Colour Stability of Strawberry Nectars

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Abstract: Strawberry nectars have increased colour stability when produced from overripe, darker, and redder strawberries, with a high anthocyanin concentration. The post-harvest storage of strawberries has been shown to develop these properties. Nectars are frequently produced from strawberries rejected for fresh sale due to poor colour, which are insufficiently ripe to produce colour-stable nectars. This study investigated post-harvest anthocyanin development in strawberries to improve the colour and colour stability of nectars, which is the first time these developments were studied for beverage production. Strawberries at five ripeness stages were stored at 20 °C for 1 and 2 days prior to nectar production. The anthocyanin content of nectars was determined by a pH-differential method, and the colour stability was tracked for 12 weeks using a consumer Acceptance Factor, derived from CIELAB colour components. The anthocyanin content and colour stability were highly correlated, and both were dependent on ripening, with larger increases observed in under-ripe strawberries, and small to no improvement in overripe samples. Stored partially coloured strawberries produced nectars with equivalent colour stability to non-stored strawberries of normal ripeness. This allowed strawberries that were previously unsuitable for both fresh sale and nectar production to be used as a feedstock for nectar production, reducing food waste.

Keywords: ripening; pelargonidin-3-glucoside; consumer acceptance; storage; shelf life

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1. Introduction

Naturally improving the colour stability of strawberry nectars is beneficial for nectar producers, as nectar colour is an important factor in the purchasing decisions of consumers [1]. Nectars are beverages that, by law, can only contain the puree of the labelled fruit, water, sugar, and acid, and so unlike similar products (such as strawberry juice drinks) colourants (for example, blackcurrant or black carrot juices) cannot be added to enhance the colour [2]. Nectar producers must then rely on natural colour enhancement through raw material selection and processing.

The likelihood of a consumer accepting the colour of a strawberry nectar has previously been quantified by the Acceptance Factor (AF), which is derived from CIELAB colour measurements. A sample with AF < 0.4 is considered unacceptable to consumers, and a sample with AF > 0.7 is considered excellent [1].

The viability of raw strawberry material for the production of colour-stable nectars can be determined by producing nectars on a small scale and calculating the AF immediately after production (AF0), and after the nectar has been stored at 20 °C for 12 weeks (AF12), to find the extent of colour degradation during storage [3]. The colour of strawberry nectars is determined by the presence of anthocyanin pigments, the most significant of which is pelargonidin-3-glucoside [4–6]. These pigments are not stable and quickly degrade from a bright red colour, which is attractive to consumers, to an undesirable brown colour. The

anthocyanin content has previously been shown to be highly correlated to the shelf life of strawberry products [3,7–9] with a higher content of anthocyanin, resulting in a better colour after storage, and consequently a longer shelf life. Increasing the concentration of anthocyanin compounds in strawberry raw material should lead to an increase in colour stability of the resultant strawberry nectar. Previous research has shown that anthocyanin content increases during ripening [10–13] and the ripeness stage of strawberries is very influential on the colour stability, with overripe strawberries producing nectars with better colour stability [14,15].

During ripening, in addition to developing in colour and anthocyanin content, strawberries become softer [12,16]. This makes them more difficult to transport, because softer fruits are more likely to be damaged, and have a shorter shelf life [17,18]. In order to maximise shelf life, strawberries for the fresh market are typically harvested before they have fully developed to their peak colour and anthocyanin content, and consequently are not at the optimal ripeness to produce nectars with the most stable colour.

In many countries, nectars are regularly manufactured from stock that has been rejected for the fresh market. Unfortunately, this results in nectar producers having little control over the cultivar and ripeness point of the fruits they receive for processing. High picking costs makes harvesting overripe fruits exclusively for nectar production prohibitively expensive. Strawberries for the fresh market are graded and sorted into classes by visual inspection, with strict criteria on superficial factors such as their size and shape. Colour is also an important factor, particularly the proportion of white skin, with strawberries with more than 20% white skin being unsuitable for fresh sale [19]. Improving the colour stability of nectars produced from these under-ripe strawberries would be advantageous to nectar producers, as it would prevent the wastage of strawberries that are currently unsuitable for both fresh sale and nectar production.

While strawberries are generally accepted to be a non-climacteric fruit [20], many studies have shown that strawberries continue to develop in both colour [21–23] and anthocyanin content after harvest [13,24,25]. The development of colour has been shown to vary based on the ripeness stage of the strawberries, with white and partially coloured fruits becoming fully red [25–27] and ripe strawberries experiencing a reduction in L* value (i.e., becoming darker) [22,28]. A redder, darker colour is an indicator that strawberry nectars would have a good colour after storage, and therefore a longer shelf life [3]. The changes in colour and anthocyanin content have been shown to vary with temperature and light, with larger changes observed in strawberries stored at higher temperatures and exposed to higher amounts of light [23,24,26,28]. Numerous studies have found that higher temperatures are necessary to develop strawberry colour in half-coloured fruits [21,25,27], although colour development has also been described at low temperature [29].

Although the development in colour and anthocyanin content mimics on-plant ripening, research indicates that other parameters do not develop in the same way as in fruit ripened on the plant. In particular, stored white strawberries did not develop the same sugar and acid contents and aroma profiles as plant-ripened strawberries [27,30], which made them unsuitable for fresh sale.

In addition to their effect on colour and colour stability, anthocyanins in strawberries act as antioxidants [31], the consumption of which has been shown to have a positive impact on human health [32]. Increasing the concentration of anthocyanins in strawberry nectars could therefore be considered as a means of enhancing the functionalisation of these beverages.

In this study, the main aim was to investigate if the previously observed development in anthocyanin content during post-harvest strawberry storage could be utilised to improve the colour and colour stability of nectars produced from these strawberries. While post-harvest colour development has been extensively studied in fresh strawberries, the potential of using these fruits in derivative products has not been previously investigated. Due to the importance of the ripening stage on nectar quality, the effects of post-harvest storage on strawberries were investigated at various stages of ripeness. Strawberries that were

unsuitable for both fresh sale and nectar production (due to being under ripe) were of particular interest. Improving these strawberries to the point that they became a suitable raw material for nectar production would reduce food waste and promote sustainability in nectar production.

2. Materials and Methods

2.1. Strawberries

Strawberries were collected and sorted into five ripeness stages, depending on their surface colour: White (1) (strawberries with no visible red/orange colour), White–Orange (strawberries that were partially white, and partially orange. Surface colour was more than 20% white, and therefore unsuitable for Class II strawberries [19]) (2), Orange (3), Red (4) (less than 10% white surface [19]) and Dark Red (5) as shown in Figure 1. Stages 1–3 are considered under ripe, stage 4 is normal ripeness, and stage 5 is overripe.

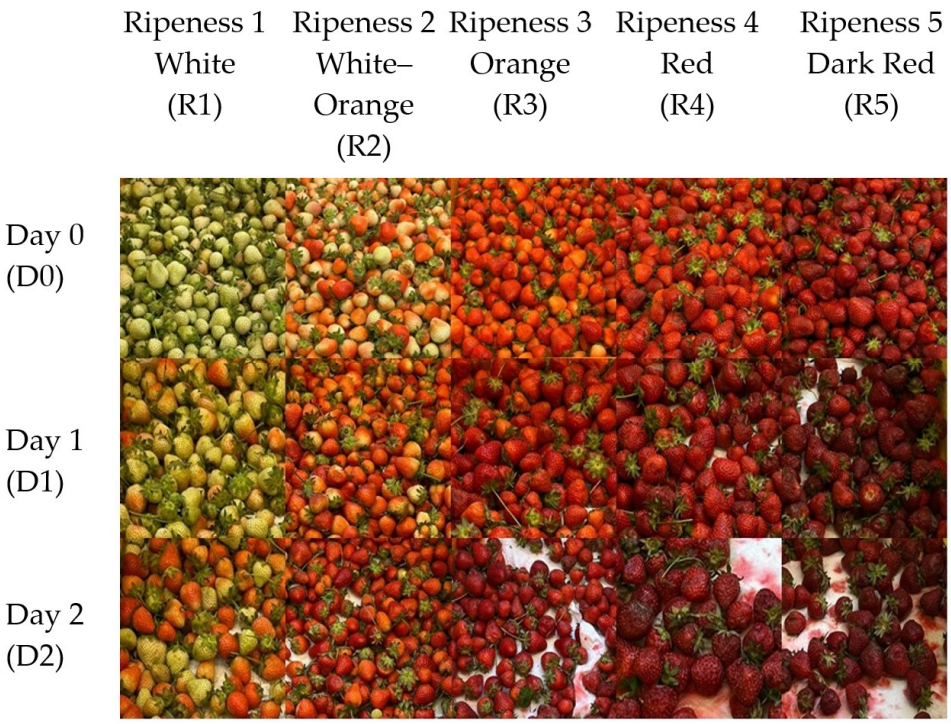

Figure 1. Strawberries of the cultivar 'Malling Centenary' sorted into ripeness grades according to surface colour on the day of picking. From left to right: White (R1), White–Orange (R2), Orange (R3), Red (R4), and Dark Red (R5). The top row shows these strawberries on the day of harvest (Day 0), the middle row 24 h later (Day 1), and the bottom row 48 h after picking (Day 2).

Six variants of strawberries from five cultivars ('Allegro', 'Sibilla', 'Magnum', 'Salsa', and 'Malling Centenary') collected from Lower Austria were separated into these ripeness stages to give a total of 30 variants on day 0. A minimum of 0.5 kg of strawberries from each variant were frozen on day on harvest (day 0 samples). Strawberries were left for 24 and 48 h at room temperature (20–25 °C) before another minimum 0.5 kg of strawberries from each variant were frozen, to give a total of 90 strawberry samples for nectar production. All strawberries were frozen at −18 °C for circa 1 month before processing.

The strawberry variants, and the nectars produced from them, were labelled according to their ripeness stage and number of days in storage (as shown in Figure 1), i.e., the nectar produced from white strawberries that were frozen on the day of picking were labelled as D0R1, white–orange strawberries frozen after 2 days of storage were labelled as D2R2, etc. These abbreviations are used throughout the paper.

2.2. Methods

2.2.1. Preparation of Nectars

Defrosting of Strawberries and Puree preparation

Frozen strawberries were defrosted for 24 h at 20 °C, and then processed into puree by a tomato mill with a 1 mm sieve (Reber, Luzzara, Italy).

Nectar Preparation

As described in Murray et al. (2023) [3], purees were mixed with water, citric acid, and sugar to produce nectar (40% puree, 15 °Brix, 7.0 g/L Titratable Acidity) and were homogenised with a hand blender (Philips, Drachten, Netherlands). In total, 140 g ± 1 g of nectar was weighed into glass jars (212 mL), sealed with a screw lid, and pasteurised (85 °C 20 min) in a water bath (Royal Catering, Berlin, Germany). Nectars were stored at 20 °C ± 3 °C in the dark.

2.2.2. Physical and Chemical Analysis

Soluble solids (°Brix) were measured with a hand-held refractometer (N-20, Brix 0~20%, ATAGO, Tokyo, Japan). pH-values were determined with a pH meter (MultilineP4, WTW, Weilheim, Germany) and a pH-electrode (SenTix 41-3, WTW, Weilheim, Germany). Titratable Acidity (TA) 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. Firmness values were obtained by a penetrometer (Mecmesin, AFG 500N, stamp 8 mm, UK) measuring 10 fresh strawberries per variant, on 2 sides (20 measurements total) and multiplied by 2 to express firmness in kg/cm^2. Weight measurements were performed by weighing 10 strawberries per variant on a balance (EW 4200-2NM, Kern & Sohn GmbH, Balingen, Germany). A digital calliper (Tchibo, Hamburg, Germany) was used to take width and length measurements of 10 strawberries per variant. Width measurements were taken at the widest point and length measurements from stem to tip.

2.2.3. 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 $C^* = \sqrt{a^2 + b^2}$ and $h^\circ = \text{atan2}(b^*, a^*)$, as previously reported [33]. The Acceptance Factor (AF) was calculated as AF $= \frac{a^*}{h}$, as previously reported [1]. All nectars were measured after pasteurisation on the day of production. Two samples were taken from each jar, which were measured in duplicate to give 4 measurements. The colour stability was quantified with different indicators of colour stability: AF12 is the AF after 12 weeks of storage at 20 °C. D12 is the difference in AF between the day of production and after 12 weeks of storage, as described in Murray et al. (2023) [3].

2.2.4. Anthocyanin Concentration

The total monomeric anthocyanin content of pasteurised nectar was determined by the pH differential method as outlined in Lee et al. (2005) [34]. The absorbance of samples in buffers with pH at 1.0 (0.025 M KCl) and 4.5 (0.4 M $CH_3CO_2Na.3H_2O$) were measured at 496 and 700 nm [35] using a spectrophotometer (Hach Lange DR 3900, Hach Lange, Düsseldorf, Germany) to obtain results as mg of pelargonidin-3-glucoside equivalents per kg by using the equation:

$$\text{TMA} = (\text{A} \times \text{Mw} \times \text{DF} \times 1000)/\varepsilon \times l \quad (1)$$

where TMA is the content of total monomeric anthocyanins, and A is the absorbance of samples [(A_{496} − A_{700} nm) pH 1.0 − (A_{496} − A_{700} nm) pH 4.5]. Mw is the molecular weight of pelargonidin-3-glucoside (433.2 g/mol), DF is the dilution factor (10 for all nectars), and

ε is the molar extinction (15,600 in pH 1 0.025 M KCl buffer [36]). l is the path-length (1 cm). Extraction and measurement were undertaken in triplicate.

2.2.5. Statistical Analysis

Statistical analyses (One-way analysis of variance (ANOVA), Tukey-HSD, paired *t*-tests, *t*-tests, Curve fitting, Regression Analysis, and Pearson Correlations) were carried out using IBM SPSS 26 (Statistical Package for the Social Sciences).

3. Results and Discussion

3.1. Colour Stability

Table 1 shows that, when averaged across all ripening stages and cultivars, the initial nectar colour (AF0) and the Acceptance Factor after 12 weeks' storage (AF12) significantly improved. These improvements increased with a longer period of time in post-harvest storage. Large standard deviations in the AF0, AF12, and the mean difference can be attributed to large differences in colour development between the different ripeness stages, as shown in Table 2. The full statistics for the paired *t*-tests for Tables 1 and 2 can be found in the Supplementary Information, Tables S1 and S2 respectively.

Table 1. Mean and standard deviation of AF0 and AF12 of nectars produced from strawberries on the day of harvest (Day 0) and after 1 and 2 days of storage, and the mean difference between the samples AF0 and AF12 and the results on Day 0.

Days in Storage Prior to Processing	Acceptance Factor of Nectars on the Day of Nectar Production (AF0)	Mean Difference with Day 0 (Paired *t*-Test)	Acceptance Factor of Nectars after 12 Weeks Storage at 20 °C (AF12)	Mean Difference with Day 0 (Paired *t*-Test)
Day 0	0.586 ± 0.303 a	-	0.356 ± 0.219 a	-
Day 1	0.691 ± 0.271 b	0.104 ± 0.091 ***	0.429 ± 0.203 b	0.073 ± 0.083 ***
Day 2	0.807 ± 0.156 c	0.221 ± 0.178 ***	0.492 ± 0.161 c	0.136 ± 0.110 ***

Different lower-case letters (vertical) illustrate significant differences (Tukey's test $p < 0.05$). *** Indicates significance at $p < 0.001$.

Table 2. Mean and standard deviation of AF0 and AF12 of nectars produced from strawberries on the day of harvest (Day 0) and after 1 and 2 days of storage, for each ripeness stage, and the mean difference between the samples AF0 and AF12 and the results on Day 0.

Strawberry Sample	Acceptance Factor of Nectars on the Day of Nectar Production (AF0)	Mean Difference with Day 0 (Paired *t*-Test)	Acceptance Factor of Nectars after 12 Weeks Storage at 20 °C (AF12)	Mean Difference with Day 0 (Paired *t*-Test)
Day 0 Ripeness 1 (D0R1)	0.057 ± 0.020 a	-	0.079 ± 0.013 a	-
Day 1 Ripeness 1 (D1R1)	0.176 ± 0.034 b	0.119 ± 0.027 ***	0.111 ± 0.023 ab	0.032 ± 0.016 ***
Day 2 Ripeness 1 (D2R1)	0.525 ± 0.081 d	0.468 ± 0.077 ***	0.224 ± 0.040 c	0.145 ± 0.037 ***
Day 0 Ripeness 2 (D0R2)	0.467 ± 0.093 c	-	0.171 ± 0.059 bc	-
Day 1 Ripeness 2 (D1R2)	0.712 ± 0.092 e	0.246 ± 0.099 ***	0.360 ± 0.112 d	0.189 ± 0.107 ***
Day 2 Ripeness 2 (D2R2)	0.836 ± 0.074 fg	0.369 ± 0.094 ***	0.478 ± 0.085 ef	0.307 ± 0.054 ***
Day 0 Ripeness 3 (D0R3)	0.743 ± 0.044 e	-	0.416 ± 0.106 de	-

Table 2. *Cont.*

Strawberry Sample	Acceptance Factor of Nectars on the Day of Nectar Production (AF0)	Mean Difference with Day 0 (Paired *t*-Test)	Acceptance Factor of Nectars after 12 Weeks Storage at 20 °C (AF12)	Mean Difference with Day 0 (Paired *t*-Test)
Day 1 Ripeness 3 (D1R3)	0.811 ± 0.044 f	0.069 ± 0.037 ***	0.502 ± 0.084 efg	0.086 ± 0.034 ***
Day 2 Ripeness 3 (D2R3)	0.869 ± 0.043 ghi	0.126 ± 0.043 ***	0.575 ± 0.073 ghi	0.159 ± 0.045 ***
Day 0 Ripeness 4 (D0R4)	0.807 ± 0.039 f	-	0.529 ± 0.124 fgh	-
Day 1 Ripeness 4 (D1R4)	0.860 ± 0.025 fgh	0.053 ± 0.023 ***	0.554 ± 0.107 fghi	0.026 ± 0.027 ***
Day 2 Ripeness 4 (D2R4)	0.885 ± 0.034 ghi	0.077 ± 0.038 ***	0.578 ± 0.079 ghi	0.050 ± 0.034 ***
Day 0 Ripeness 5 (D0R5)	0.858 ± 0.047 fgh	-	0.585 ± 0.124 ghi	-
Day 1 Ripeness 5 (D1R5)	0.893 ± 0.040 hi	0.036 ± 0.026 ***	0.616 ± 0.109 i	0.031 ± 0.043 **
Day 2 Ripeness 5 (D2R5)	0.921 ± 0.055 i	0.063 ± 0.035 ***	0.605 ± 0.101 hi	0.020 ± 0.051 ns

Different lower-case letters (vertical) illustrate significant differences (Tukey's test $p < 0.05$). *** and ** and ns indicate significance at $p < 0.001$, $p < 0.01$, and no significance, respectively.

The varied development in colour between the different ripeness grades are visualised in the boxplots in Figure 2.

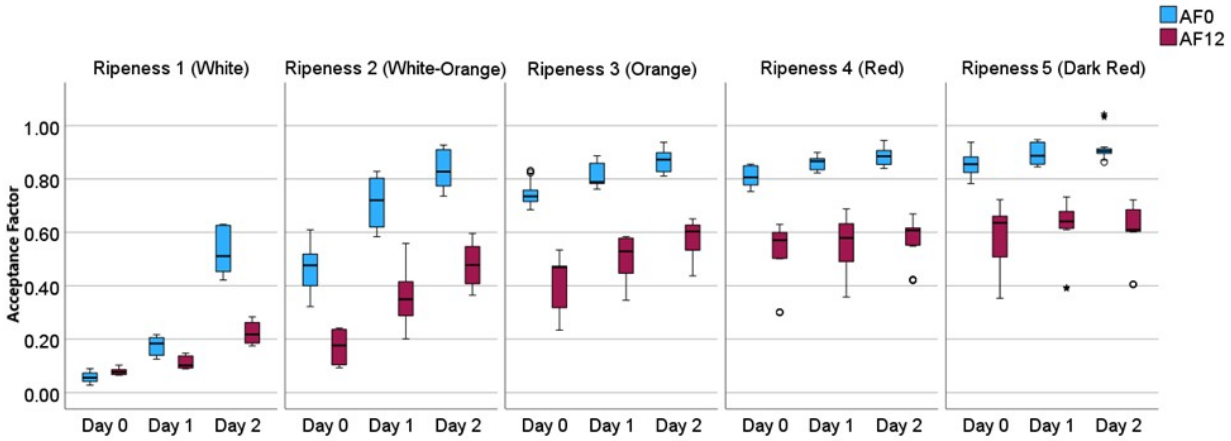

Figure 2. Boxplots showing the change in the Acceptance Factor when initially produced (AF0), and after 12 weeks storage, when made from strawberries that were fresh (Day 0), and stored for 24 h (Day 1) and 48 h (Day 2). Circles represent mild outliers (values more than 1.5 times the interquartile range (IQR) below Q1 or above Q3) and asterisks represent extreme outliers (values more than 3.0 times the IQF below Q1 or above Q3).

3.1.1. Ripeness Stage 1 (White)

Nectars produced from D2R1 strawberries had the largest improvement in AF0. After 2 days, all nectars had developed in colour to the point that they had an Acceptance Factor above 0.4 (i.e., an acceptable colour to consumers). This is in agreement with previous studies that found colour could develop in white strawberries [21]. The colour of nectars produced from the stored white strawberries was not stable, and quickly degraded below AF 0.4, resulting in an average improvement in AF12 that was less than half the

improvement in AF0. As the colour continuously improved during storage, it is possible that with additional time in storage, white strawberries could further develop to the point where they could produce nectars of an acceptable colour. However, findings from previous studies [29] suggest that white strawberries would never become suitable for nectar production, and so white strawberries should not be stored for this purpose.

3.1.2. Ripeness Stage 2 (White–Orange)

R2 were the most promising in using post-harvest storage to improve the colour stability, as nectars produced from D2R2 had the largest increase in AF12 of all nectars produced. For these samples, there were similar increases in AF0 and AF12, suggesting that unlike the D2R1 samples, the improvements in colour in D2R2 were relatively stable. Immediately after harvest, AF0 was only just above the level of acceptability (0.4), whereas after 2 days of storage the colour had increased to an excellent AF0 value (above 0.7). AF0 improved to the point that there was no significant difference between nectars from D2R2 and nectars produced from D0R4 (ripe strawberries on the day of harvest) ($t(46) = 1.7$, $p = 0.097$). This improvement in colour resulted in an average AF12 that was still above the level of consumer acceptability (0.4), with no significant difference between nectars produced from D2R2 strawberries and nectars produced from the D0R4 strawberries ($t(46) = -1.8$, $p = 0.083$).

3.1.3. Ripeness Stage 3 (Orange)

Nectars produced from D1R3 and D2R3 strawberries also showed significant improvements in both AF0 and AF12. The increase in AF12 was higher than that in AF0, so the development in colour was particularly stable. Unlike D0R1 or D0R2, nectars produced from D0R3 had an AF0 that was acceptable, and as a result these samples required less time in storage to develop an AF0 that was equally acceptable as D0R4. Nectars produced from D1R3 did not significantly differ from those produced from D0R4, both on the day of production AF0 ($t(46) = 0.4$ $p = 0.738$), or after 12 weeks AF12 ($t(46) = -0.9$ $p = 0.354$). Moreover, nectars produced from D2R3 had significantly surpassed the AF0 of nectars produced from D0R4 ($t(46) = 5.23$ $p < 0.001$), although the AF12 had not $t(46) = 1.7$ $p = 0.091$). Strawberries of ripeness 3 required only 1 day of storage to produce nectars that were as stable as nectars made from those picked at a normal ripeness. Minimising the duration of storage decreases the risk of spoilage, and cuts storage expenses. It is therefore advantageous to have strawberries that yield acceptable products after only 1 day of storage, in order to mitigate these risks.

3.1.4. Ripeness Stage 4 (Red)

Nectar produced from D1R4 and D2R4 had small but significant increases in the mean difference for both AF0 and AF12, compared to D0R4. The increase in AF0 was larger than that in AF12, implying that this colour enhancement may not be as stable as the increases observed in R2 and R3. The minimal increases in colour development suggest that post-harvest storage of this ripeness grade does not provide sufficient benefits to justify the cost of storage, and the risk of spoilage associated with storage.

3.1.5. Ripeness Stage 5 (Dark Red)

Nectars produced from D2R5 showed no significant increase in AF12, compared to the non-stored D0R5 samples. There were also only minimal increases in the AF0 of D1R5 and D2R5, and the AF12 of D1R5. This result suggests that there is little to no tangible advantage of keeping overripe fruits in post-harvest storage, as the observed improvements were negligible. Furthermore, the improvement in AF12 seen in D1R5, where no improvements were observed in D2R5 suggests that longer storage periods of these samples can have a detrimental effect. Therefore, it is not recommended that these samples are held in post-harvest storage prior to processing.

3.1.6. Cultivar Effects

Multiple cultivars were assessed to confirm that the effect of post-harvest storage was consistent across a range of cultivars. Nectar producers are often unable to select which cultivar they receive for processing, as they are obliged to use rejected stock from the fresh market. In many cases, nectar producers will not know which cultivars they are processing. It was therefore important to verify that any development in colour stability was universal across all cultivars. If colour developments were only present in some cultivars, it would pose too great of a risk for nectar processors to utilise post-harvest storage. The mean difference between D0 variants, and the variants of D1 and D2, are given in the Supplementary Information Table S4. All cultivars showed significant increases in AF0, AF12, and anthocyanin content in ripeness stages 1, 2, and 3, after both 1 and 2 days of storage. This result is encouraging as it suggests that this behaviour would be expected in many cultivars, and nectar producers would not need to be concerned that storage of these under-ripe ripeness stages would not provide a benefit to colour stability. Ripeness stages 4 and 5 were less consistent, with some cultivars increasing in AF12, while other cultivars showed decreasing AF12, or no difference at all. The fact that these cultivars did not universally increase in AF12 is further evidence that strawberries of these ripeness stages should not be stored, as while storage might lead to an improvement in some cultivars, it could have a detrimental effect in others. This variability means that storing these ripeness grades would pose too great a risk for nectar producers, especially when processing unknown cultivars.

3.2. Anthocyanin Content

3.2.1. Anthocyanin Development during Storage

The anthocyanin content of the nectars made from the different variants of strawberries increased significantly during storage, as shown in Table 3. These findings were consistent with previous research, which also found that strawberry anthocyanin content increased during storage [13,24,25]. The mean difference between the nectars made from non-stored and stored fruits was significant, but with large standard deviations.

Table 3. Mean and standard deviation of anthocyanin content of nectars produced from strawberries on the day of harvest (Day 0) and after 1 and 2 days of storage, and the mean difference between the samples AF0 and AF12 and the results on Day 0.

Days in Storage Prior to Processing	Total Monomeric Anthocyanin Content [mg/kg pg-3-glu eqv]	Mean Difference with Day 0 (Paired *t*-Test)
Day 0	86.5 ± 75.9 a	-
Day 1	103.2 ± 68.0 ab	16.7 ± 21.0 ***
Day 2	120.1 ± 57.9 b	33.6 ± 33.2 ***

Different lower-case letters (vertical) illustrate significant differences (Tukey's test $p < 0.05$). *** Indicates significance at $p < 0.001$.

The development of anthocyanin content was markedly different between the different ripeness stages, which is shown in Table 4.

White strawberries increased from close to no content in D0R2, to a level where D2R1 had a higher content than that found in D0R2. In the non-stored white strawberries, there was either very little or no anthocyanin content, which demonstrates that white strawberries can develop anthocyanin content similar to strawberries that have already started developing visible surface colour, even when the content of anthocyanin in the initial samples is negligible.

R2 had the largest increase in anthocyanin content, steadily rising over the 2 days until it had surpassed the content found in non-stored fully coloured fruits (D0R3).

Anthocyanin content also increased significantly during storage of R3 strawberries. The anthocyanin content of D1R3 had no significant difference to the anthocyanin content of D2R2. This reinforces the findings from the colour results in Section 3.1, that R3 should

be held for only 1 day (rather than the 2 days recommended for R2) in order to achieve a good compromise between anthocyanin and colour development and other quality parameters. In comparison, nectars produced from D1R4 and D2R4 had a negligible increase in anthocyanin content and no significant difference in anthocyanin content could be found between D0R5, D1R5, and D2R5.

Table 4. Mean and standard deviation of anthocyanin content of nectars produced from strawberries on the day of harvest (Day 0) and after 1 and 2 days of storage for each ripening stage, and the mean difference between the samples' AF0 and AF12 and the results on Day 0.

Strawberry Sample	Total Monomeric Anthocyanin Content [mg/kg pg-3-glu eqv]	Mean Difference with Day 0 (Paired *t*-Test)
Day 0 Ripeness 1 (D0R1)	0.9 ± 1.2 a	-
Day 1 Ripeness 1 (D1R1)	7.7 ± 2.8 ab	6.7 ± 2.5 ***
Day 2 Ripeness 1 (D2R1)	33.4 ± 7.9 b	32.4 ± 7.2 ***
Day 0 Ripeness 2 (D0R2)	23.6 ± 8.4 ab	-
Day 1 Ripeness 2 (D1R2)	66.9 ± 24.9 c	43.3 ± 22.1 ***
Day 2 Ripeness 2 (D2R2)	102.9 ± 20.8 de	79.3 ± 18.5 ***
Day 0 Ripeness 3 (D0R3)	77.4 ± 16.3 cd	-
Day 1 Ripeness 3 (D1R3)	108.5 ± 14.1 ef	31.1 ± 7.0 ***
Day 2 Ripeness 3 (D2R3)	128.0 ± 18.1 efg	50.5 ± 19.8 ***
Day 0 Ripeness 4 (D0R4)	137.5 ± 19.5 fg	-
Day 1 Ripeness 4 (D1R4)	142.3 ± 19.0 g	4.7 ± 5.35 **
Day 2 Ripeness 4 (D2R4)	146.7 ± 23.3 g	9.2 ± 11.9 ns
Day 0 Ripeness 5 (D0R5)	193.2 ± 50.8 h	-
Day 1 Ripeness 5 (D1R5)	190.7 ± 47.3 h	−2.5 ± 10.8 ns
Day 2 Ripeness 5 (D2R5)	189.6 ± 44.5 h	−3.5 ± 14.6 ns

Different lower-case letters (vertical) illustrate significant differences (Tukey's test $p < 0.05$). *** and ** and ns indicate significance at $p < 0.001$, $p < 0.01$ and no significance respectively.

Nectars produced from overripe strawberries had a significantly higher anthocyanin content than all other nectars, even those produced from stored strawberries of ripeness 4. Overripe strawberries did not develop in anthocyanin content during storage, with no significant difference between stored and fresh strawberries. In the stored R5 samples, a small (although insignificant) decrease in anthocyanin content was observed. This is potentially due to anthocyanin degradation during strawberry storage. These results serve as further evidence that both ripe and overripe strawberries should not be stored, as the R4 value never reached the anthocyanin content of overripe strawberries, even after storage. If maximising anthocyanin content is the aim of nectar producers, strawberries

should be left on the plant to develop further into the overripe stage. However, if the strawberries have already been picked prematurely (before they are fully ripe and have not reached an acceptable level of anthocyanin content) then post-harvest storage can greatly improve the anthocyanin content, thereby functionalising the nectars by increasing the antioxidant content.

3.2.2. Anthocyanin Content and Colour Stability

Anthocyanin content has previously been shown to be highly correlated with colour stability [3], and this work supported those findings.

Figure 3 shows the relationship between the anthocyanin content and AF12. There was a strong and significant correlation (r = 0.893 $p < 0.001$), as well as a significant regression ($R^2 = 0.798$, $F(1,268) = 1056.7$, $p < 0.001$). This means that 79.8% of the variance in AF12 could be explained by the anthocyanin content, and the regression coefficient (B = 0.003, 95% CI [0.002, 0.003]) indicated that an increase in anthocyanin content of 1 mg/kg increases AF12 by 0.003. This confirms that the anthocyanin content has a large effect on the colour of nectars after storage, and so the shelf life of nectars is also increased. This study also demonstrated that anthocyanin determination by pH-differential methods could be used as a method to predict the colour stability of nectars, by quickly measuring their anthocyanin content on the day of production, without the need for expensive equipment and expertise required for HPLC measurements.

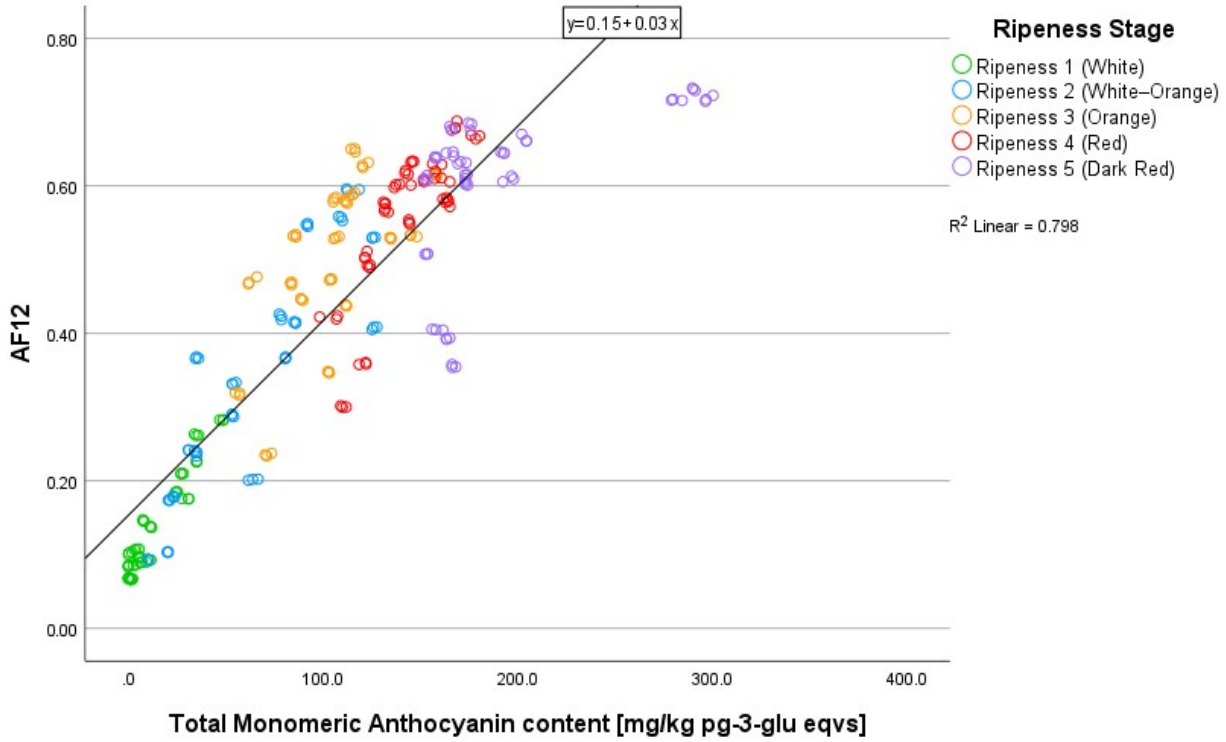

Figure 3. Nectars' total monomeric anthocyanin content, in mg/kg, expressed as pelargonidin-3-glucoside equivalents, plotted against the corresponding nectars' acceptance factor after 12 weeks of storage at 20 °C.

Despite the strong relationship between the anthocyanin content and the AF12, differences were evident between the development of the anthocyanins during storage and changes in colour stability. While the nectars from D2R2 had no significant difference in colour stability compared to those produced from D0R4 (Section 3.1.2), the anthocyanin content of the nectar made from D0R4 was still significantly higher than that in the nectar produced from strawberries of D2R2 ($t(34) = -5.1$, $p < 0.001$).

3.3. Physical Parameters

3.3.1. Total Soluble Solids, Titratable Acidity, firmness, and pH

The average values of the Total Soluble Solids (TSSs) (°Brix), Titratable Acidity (TA)(g/L), firmness, and pH are summarised in Table 5. Full statistics for the paired *t*-tests can be found in the Supplementary Information Table S3.

Table 5. Mean and standard deviation of the Total Soluble Solids (in °Brix), the Titratable Acidity (in g/L), and the pH of purees of strawberries stored for 0, 1, and 2 days for each ripening stage, and the mean difference between stored and day 0. The firmness of these strawberries prior to freezing is also shown.

Strawberry Sample	Total Soluble Solids (TSSs) [°Brix]	Mean Difference with Day 0 (Paired *t*-Test)	Titratable Acidity (TA) [g/L]	Mean Difference with Day 0 (Paired *t*-Test)	pH	Mean Difference with Day 0 (Paired *t*-Test)	Firmness [kg/cm^2]	Mean Difference with Day 0 (Paired *t*-Test)
Day 0 Ripeness 1 (D0R1)	7.0 ± 1.2 a	-	11.6 ± 3.0 ef	-	3.3 ± 0.1 abc	-	1.85 ± 2.03 c	-
Day 1 Ripeness 1 (D1R1)	7.1 ± 1.4 a	0.08 ± 0.33 ns	11.9 ± 2.9 ef	0.26 ± 1.1 ns	3.3 ± 0.1 abc	0.50 ± 1.1 ns	1.63 ± 1.86 c	−0.21 ± 0.91 ns
Day 2 Ripeness 1 (D2R1)	7.5 ± 1.4 a	0.45 ± 0.32 *	13.5 ± 4.7 f	1.95 ± 2.55 ns	3.2 ± 0.2 a	0.43 ± 1.06 ns	0.95 ± 0.68 b	−0.89 ± 0.53 *
Day 0 Ripeness 2 (D0R2)	7.2 ± 1.3 a	-	10.1 ± 2.5 cde	-	3.3 ± 0.1 ab	-	0.50 ± 0.46 a	-
Day 1 Ripeness 2 (D1R2)	7.3 ± 1.6 a	0.12 ± 0.56 ns	10.0 ± 2.6 cde	−0.06 ± 0.83 ns	3.3 ± 0.1 abc	0.05 ± 0.14 ns	0.51 ± 0.30 a	0.01 ± 0.21 ns
Day 2 Ripeness 2 (D2R2)	7.9 ± 1.8 a	0.65 ± 0.77 ns	9.1 ± 2.0 de	0.68 ± 0.90 ns	3.3 ± 0.0 ab	−0.003 ± 0.17 ns	0.47 ± 0.30 a	−0.03 ± 0.29 ns
Day 0 Ripeness 3 (D0R3)	7.1 ± 1.3 a	-	8.3 ± 2.0 abc	-	3.3 ± 0.1 abc		0.24 ± 0.12 a	-
Day 1 Ripeness 3 (D1R3)	7.2 ± 1.2 a	0.02 ± 0.45 ns	8.6 ± 2.1 abcd	0.18 ± 0.25 *	3.4 ± 0.1 de	0.14 ± 0.16 ns	0.34 ± 0.18 a	0.10 ± 0.04 **
Day 2 Ripeness 3 (D2R3)	7.3 ± 1.3 a	0.17 ± 0.61 ns	9.1 ± 2.0 bcd	0.86 ± 0.30 **	3.4 ± 0.1 cd	0.09 ± 0.16 ns	0.28 ± 0.17 a	0.04 ± 0.12 ns
Day 0 Ripeness 4 (D0R4)	7.8 ± 1.0 a	-	6.9 ± 1.6 ab	-	3.5 ± 0.2 efg	-	0.19 ± 0.10 a	-
Day 1 Ripeness 4 (D1R4)	7.9 ± 1.1 a	0.13 ± 0.30 ns	7.3 ± 1.3 ab	0.35 ± 0.37 ns	3.4 ± 0.1 efg	0.0008 ± 0.14 ns	0.23 ± 0.13 a	0.04 ± 0.06 ns
Day 2 Ripeness 4 (D2R4)	7.9 ± 1.0 a	0.12 ± 0.60 ns	8.6 ± 1.8 abcd	1.65 ± 1.08 *	3.4 ± 0.1 bcd	−0.16 ± 0.17 ns	0.21 ± 0.19 a	0.02 ± 0.14 ns
Day 0 Ripeness 5 (D0R5)	7.8 ± 1.4 a	-	6.4 ± 1.6 a	-	3.6 ± 0.2 f	-	0.16 ± 0.07 a	-
Day 1 Ripeness 5 (D1R5)	8.1 ± 1.7 a	0.13 ± 0.48 ns	7.4 ± 2.8 ab	1.00 ± 1.47 ns	3.6 ± 0.1 ef	−0.06 ± 0.18 ns	0.16 ± 0.10 a	0.004 ± 0.03 ns
Day 2 Ripeness 5 (D2R5)	8.0 ± 1.9 a	0.12 ± 0.85 ns	7.9 ± 1.6 abc	1.41 ± 0.78 **	3.5 ± 0.1 def	−0.17 ± 0.22 ns	-	-

Different lower-case letters (vertical) illustrate significant differences (Tukey's test $p < 0.05$). **, * and ns indicate significance at $p < 0.01$, $p < 0.05$, and no significance, respectively.

On the day of harvest, D0R5 had the highest TSS and lowest TA values, and D0R1 had the lowest TSS and highest TA values. This is in agreement with previous studies, which found that TSS increases and TA decreases with increasing ripening stage [12,37]. There was no significant increase in the TSS value after storage. Conversely, TA (in some cases)

exhibited significant increases, consistent with previous findings in the literature that TA increases during storage [13]. This increase could be attributed to strawberry respiration during storage. Respiration has been shown to reduce the weight and water content in fruits, and thereby slightly increases the proportion of acid and sugar [13].

Strawberries at R1 were far firmer than all the other ripeness stages, and despite becoming significantly less firm after 2 days, these samples still remained firmer than strawberries of all other ripeness stages. It was not possible to gain firmness data on R2R5, as these strawberries became too soft to give accurate measurements and were incredibly difficult to handle. This further confirms that storing overripe strawberries is not a good strategy for improving nectar quality, as in addition to only having small, or negligible, changes in colour stability, the quality of overripe strawberries was markedly compromised by storage. In contrast, the strawberries D2R2 and D2R3 were more firm than D0R4 or D0R5, and so retained the ease of handling that is desired in less ripe strawberries. Increased firmness makes them easier to process than normal and overripe strawberries, and more resistant to damage.

3.3.2. Size and Weight

The size and weight of the strawberries of the different ripeness stages at D0 are summarised in Table 6. The weight and size increased with the ripening stage. Larger strawberries are favoured by the industrial harvesters, as it takes less time to pick larger strawberries to achieve a desired weight. The disadvantages of the small size of under-ripe strawberries would be exacerbated by previous research that found that the respiration of strawberries results in further weight reduction during storage [13]. Therefore, picking strawberries that were already smaller than strawberries from a standard harvest, and then storing them would further reduce fruit size. This implies that intentionally picking under-ripe stock with the intention of storing them until they are fully red would not be an advisable strategy for strawberry harvesters. Nonetheless, smaller strawberries cannot be marketed as top-class fresh strawberries, making it more probable that small, under-ripe strawberries will be used as the feedstock for nectar production. In this context, enhancing the colour of small, under-ripe fruits would be particularly advantageous for nectar producers.

Table 6. The average weight (in g), length (measured from stem to tip in mm), and width (measured at the widest point of the strawberry) of strawberries of different ripeness stages.

Ripeness Grade	Weight [g]	Length [mm]	Width [mm]
Ripeness 1 White (D0R1)	6.4 ± 2.9 a	22.5 ± 5.2 a	21.2 ± 4.1 a
Ripeness 2 White/Orange (D0R2)	8.8 ± 3.6 ab	24.4 ± 5.6 ab	23.6 ± 4.2 ab
Ripeness 3 Orange (D0R3)	11.3 ± 5.8 bc	27.0 ± 5.5 bc	26.2 ± 6.2 bc
Ripeness 4 Red (D0R4)	13.5 ± 6.3 cd	29.1 ± 5.2 c	28.4 ± 6.6 cd
Ripeness 5 Dark Red (D0R5)	14.2 ± 5.1 d	29.6 ± 5.4 c	29.4 ± 5.4 d

Different lower-case letters (vertical) illustrate significant differences (Tukey's test $p < 0.05$).

4. Conclusions

The impact of post-harvest storage on the colour, physical, and chemical parameters of strawberries was heavily influenced by their ripeness stage. The colour stability and anthocyanin content of strawberry nectars produced from under-ripe strawberries (ripeness stages 1–3) were improved by post-harvest storage of these fruits. The colour of nectars produced from orange strawberries (ripeness 3) that had been stored for 1 day and partially

white strawberries (ripeness 2) stored for 2 days were as acceptable to consumers as nectars produced from non-stored strawberries of a normal ripeness (ripeness 4). These strawberries remained firmer after storage than non-stored ripeness 4 strawberries, and so retained the quality and ease of handling that necessitates the premature harvest of strawberries. While the post-harvest development of colour and anthocyanin content in strawberries has been extensively covered in the literature, this is the first time that these developments have been studied for practical use in a derivative product. The colour development of partially white strawberries is particularly significant, as strawberries with a white surface area greater than 20% cannot be sold in the fresh market. Without storage, these strawberries yielded nectars with a completely unacceptable colour. Therefore, post-harvest storage allows strawberries that would otherwise go to waste to be utilised in nectar production, reducing food waste and providing an economic advantage to nectar producers.

The anthocyanin content was highly correlated to the colour acceptance of nectars after storage (AF12), to the extent that measuring anthocyanin content on the day of nectar production provided a prediction of how acceptable the nectar colour would be to consumers after 12 weeks of nectar storage. As such, consistent with the results from the colour measurements, under-ripe strawberries exhibited the largest increases in anthocyanin content during storage. The largest increase occurred in strawberries that were partially white, partially coloured (Ripeness 2), and stored for 2 days. Strawberries that were of normal ripeness (Ripeness 4) and overripe (Ripeness 5) either decreased in anthocyanin content or had very small or negligible increases during storage. Despite the absence of an increase in anthocyanin content during storage, nectars produced from overripe strawberries had the highest anthocyanin content—surpassing that of any nectar produced from less ripe stored strawberries—and as such demonstrated high colour stability. It is therefore recommended that ripe and overripe strawberries are not to be stored, as the minimal enhancements in colour stability and anthocyanin content are outweighed by costs and risks associated with storage. Moreover, if strawberries are deliberately harvested for nectar production, it is preferable to allow them to ripen on the plant until they become overripe, rather than harvesting them prematurely and then storing them. However, if strawberries have been picked at a lower ripeness point, post-harvest storage can enable their use in nectar production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages10020036/s1. Table S1: Full statistics for paired *t*-tests for the AF0, AF12, and Anthocyanin Content across all ripeness stages. Table S2: Full statistics for paired *t*-tests for the AF0, AF12, and Anthocyanin Content also separated by ripeness stage. Table S3: Full statistics for the paired *t*-test statistics for the TSS, TA, pH, and firmness. Table S4: The average increase (as determined by a paired *t*-test) in AF0, AF12, and Total Monomeric Anthocyanin Content [mg/kg pg-3-glu eqv] for each cultivar at each ripeness stage, between non-stored samples (Day0) and after 1 and 2 days of post-harvest storage.

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